Pyrethroid Analysis for Maine Board of Pesticides Control

Performed by Larry LeBlanc and Brian Perkins at the University of Maine Food and Chemical Safety Laboratory, Orono, Maine, in January–April 2007.

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Activities:

Supplies ordered:

Solid phase extraction cartridges for sample cleanup:
- Envi-carb/PSA mix – ordered from Supelco
- Florisil columns: used JT Baker columns already on-hand in Food Chemistry and Safety Laboratory

Copper powder (for removal of sulfur from sediment extracts): ordered from Fisher Scientific

Sohxlet thimbles: ordered from Fisher Scientific

Received from EPA National Repository, analytical standards:

Cypermethrin:	99.5% purity
Esfenvalerate:	98.7% purity
Bifenthrin:	98.8% purity
Cyfluthrin:	49.2% purity (not used)
Permethrin:	49.5% purity (not used)

Analytical standards from sigma-aldrich

Cyfluthrin:	250 mg, 98.3% purity
Cypermethrin:	250 mg, 95.5 % purity
Permethrin:	250 mg, 98.7% purity

Octachloronaphthalene: (ordered from Ultra Scientific) 99.0% purity: added as an internal standard

Sample Inventory:

2 jars: Coyle St. near pipe	sample ID 784 9/26/06
2 jars: Coyle St. 2	sample ID 785 9/26/06
Empty jar: trip blank	
2 jars: Randall St. 1	Sample ID 786 9/26/06

2 jars: Randall St. 2 Sample ID 787 9/26/06
2 jars: Randall St. 3 Sample ID 788 9/26/06
2 jars: Randall St. 4 Sample ID 789 9/26/06

Method Development:
January 2007, February 2007. Method development proceeds. Spiked standards are placed through solid phase extraction cleanup columns – both the envi-carb/PSA mix (column type #1) and the florisil (column type #2), based upon a method described by You (2006)
Good recovery (> 85%) obtained from standard spikes, when
Column type #1 collected fraction = 7 mL of 30% methylene chloride in hexane
Column type #2: collected fraction = 10 mL of 10-% methylene chloride in hexane

Standard mixes of pyrethroid analytes, internal and surrogate standards shot on the GC/MS. A selected ion monitoring (SIM) method developed, whereby only certain mass fragments are collected, that are representative of each compound. This increases the instrumental detection by decreasing the background signal (eliminates interfering ions).

Extractions begun in March, 2007. Sample numbers 784, 785, 787 were sieved through a 2 mm mesh brass sieve to remove large amount of plant detritus present in samples.
Sample notes:
Sample # 784 was very muddy
Sample # 786 was exceptionally sandy. Other samples were a mix of sand interspersed with fine-grained detritus (could by called muddy sand). This can be quantified by grain-size analysis (beyond the scope of this study)

METHODS
Percent moisture was determined on all samples, by weighing a small amount (approx 5 grams) of wet sediment, drying the sample and reweighing.
The ratio of dry weight /wet weight varied from 0.4 – 0.49, with the exception of sample 786 (sandy sample) which had a dry/wet ratio of 0.79.

Sample Extraction: Approximately 15 grams dry weight of sediment per sample was used. Duplicate samples were analyzed for each station.

Samples were extracted by sohxlet extraction, using 250 mL of 50:50 methylene chloride:acetone. Pre-combusted sodium sulfate was added to the extracts to remove water. Samples were reduced to approximately 5 mL by rotary evaporation. Copper powder was activated by exposure to HCl, then rinsed with DI water, acetone and methylene chloride. This was added to each extract in order to remove elemental sulfur (in sediments is of the form S8), which interferes with the instrumental analysis. Extracts were reduced in volume to 1mL, and solvent-exchanged to hexane.

Extract cleanup: Sediment is one of the hardest matrices for organic analysis, because of large amount of co-extractives, requiring extensive cleanup of the extracts. Extracts

were cleaned up by passage through solid phase extraction columns (Enviro-carb/PSA mixed bed, 500 mg, Supelco, Bellefonte, PA). Analytes were eluted with 6 mL of 30% methylene chloride in hexane. These extracts were reduced to 0.5 mL and injected onto an Agilent 6890/5730 GC/MS system. In the event that sample extracts were still colored after passage through cleanup column #1 (Envi-carb/PSA), extracts were placed through a second solid phase extraction cleanup column consisting of 0.5 g of florisil. Extracts were eluted with 20% methylene chloride in hexane, reduced to 0.54 mL and shot again.

Instrumental analysis: All samples were analyzed by gas chromatography/mass spectroscopy (GC/MS), in full scan mode (collecting all ions between 50-460 amu) as well as in selected ion monitoring (SIM) mode. From the full scan analysis one obtains a 'mass fingerprint' of the analyte, which can be compared to a standard injection in order to positively identify the compound. In SIM mode, only selected ion fragments somewhat unique to the analyte are collected. This removes a great deal of background interference, produces a cleaner chromatogram, and increases sensitivity by at least a factor of 10. Analytes and internal and surrogate standards are listed in Table 1, along with analyte retention times, and ions used for quantification and confirmation.

Table 1. List of pyrethroid analytes, internal and surrogate standards, retention times and quantification ions for GC/MS analysis

	Retention Time (min)	Quant Ion	Confirmatory Ion 1	Confirmatory Ion 2
tetrachlroroxylene[1]	8.8	244	208	171
bifenthrin	23.96	244	208	171
l-cyhalothrin	24.83	197	208	181
permethrin 1	25.42	183	163	163
permethrin 2	25.56	183	281	165
OCN[2]	26.43	404	332	262
cyfluthrin 1	25.98	206	163	165
cyfluthrin 2	26.025	206	163	165
cyfluthrin 3	26.2	206	163	165
cypermethrin 1	26.29	181	163	281
cypermethrin 2	26.43	181	163	281
cypermethrin 3	26.52	181	163	281
decachlorobiphenyl[1]	26.93	498	428	356
esfenvalerate	27.66	181	152	125

[1]Compound added as an internal standard, for quantification purposes

[2]Compounds added as surrogate standards, for calculating recoveries

RESULTS and DISCUSSION:

There was no clear evidence for the presence of pyrethroids in these samples. Despite extensive cleanup, there still was significant matrix present in these extracts. There was not enough resources available to pursue more extensive cleanup options at this time. The cleanup method used was a brand new method, not yet published, but presented by the researcher (Jing You, Southern Illinois University at Carbondale) at the 2006 national meeting of the Society of Environmental Toxicology and Chemistry,

attended by L. LeBlanc. While marked improvement was seen in sample cleanup, we feel that with time and resources we could improve this.

As already mentioned, analysis by selected ion monitoring (SIM) allowed for the removal of much of this matrix interference. Samples were run twice, in full scan mode and again in SIM mode, to achieve maximum sensitivity and selectivity (SIM), while retaining the ability to confirm the presence of the analytes based upon the 'mass fingerprint' of the compound (full scan).

Pyrethroid compounds should be considered to be below detection (i.e., not detected) in these sediments. Detection limits are estimated to range between 1-10 ng/g, depending on the specific analyte. This is based upon dilute standard injections on the instrument, and a cutoff of 3x the signal:noise ratio of the instrument. Cyfluthrin and cypermethrin have the highest detection limits (approximately 10 ng/g), because the mass of these compounds is spread over multiple peaks (i.e., there are several isomers for each compound) which do not resolve to baseline, and often exhibit tailing.

In a few samples (station 784, station 785, 788,789), peaks were present in the chromatogram that resembled permethrin and bifenthrin. However the estimated concentrations of these "mystery peaks" (0.005-0.006 ng/g) are far below the limits of detection of this method and any other method known to L. LeBlanc and so should be considered as not-detected. Other evidence to discount these peaks is that the 'mass fingerprint' did not adequately match the standard, and the compound retention time (another method of peak identification having to do with when the compound elutes off the GC column and enters the mass spectrometer portion of the instrument) does not match.

To further investigate the 'true identity' of these peaks is beyond the scope of this study, and would require further method development in order to produce an extract completely free of matrix (background) interference. Even with this added effort, it is quite possible that, if indeed extremely trace concentrations are present, the resulting concentrations would be below the method detection limits and so 'legally' would have to be labeled as not detected.

This analytical method can be improved in two ways.
1.) Experiment with different solvent systems for extracting sediments (such as acetonitrile:water or methanol:water). These solvents, used in conjunction with alternative extraction methods (such as accelerated solvent extraction, which performs extractions at elevated pressures and temperatures) may leave behind some of the interfering co-extractives while isolating the analytes of interest. To investigate this would require some months of research and development effort.

2.) Experiment with alternative cleanup columns. In addition to the cleanup columns listed above, selected extracts were put through alumina columns and cation-exchange columns to test whether extracts could be cleaned up further (based upon color removal). Initial results were not compelling, although further work needs to be done in this area.

Finally, separation based upon molecular size (using gel permeation chromatography) may also yield a cleaner extract.