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EMON-SM-52-9
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Title: Analysis of Pyrethroids in Sediment

1. Scope:

   This section method (SM) documents selective pyrethroids analysis in sediment and is followed by all authorized EMON personnel.

2. Principle:

   The SM describes the method for determination of resmethrin, bifenthrin, fenpropathrin, lambda cyhalothrin epimer, lambda cyhalothrin, permethin cis, permethrin trans, cyfluthrin, cypermethrin, fenvalerate/ esfenvalerate and deltamethrin in sediment. The samples are homogenized and extracted with 1:1 acetone/hexane by shaking on an obitial shaker. The extracts are cleaned with florisil before being analyzed with a gas chromatography equipped with electron capture detector. Two columns of different polarity were used for confirmation of the analytes. The msd is used for the analysis of remethrin. Further confirmation was obtained using the msd in cases where the concentration was high enough. The msd is unable to see all the compounds at the 1 ppb reporting limits.

3. Safety:

   3.1 All general laboratory safety rules for sample preparation and analysis shall be followed.

   3.2 Acetone and hexanes are flammable and toxic solvents; they should be handled with care in a ventilated area.

4. Interferences:

   The electron capture detector (ECD) is not truly an element specific detector, it will also respond to compounds containing S, NO_2 or conjugated C=O functional groups.

5. Apparatus and Equipment:

   5.1 Shaker, (Lab-Line Force Orbital Air Shaker or equivalent)
   5.2 Rotary Evaporator (Buchi/Brinkman or equivalent)
   5.3 Nitrogen evaporator (Meyer N-EVAP Organomation Model #112 or equivalent)
   5.4 Balance, (Mettler PC 4400 or equivalent)
   5.5 Vortex-vibrating mixer

5.6 Gas Chromatograph (GC) equipped with ^{63}Ni ECD detectors
5.7 Gas Chromatograph equipped with a mass selective detector (MSD)

6. Reagents and Supplies:

6.1	Bifenthrin	CAS#42576-02-3
6.2	Fenpropathrin	CAS#39515-41-8
6.3	Lambda cyhalothrin epimer	CAS# unknown
6.4	Lambda cyhalothrin	CAS#91465-08-06
6.5	Permethrin cis	CAS#54774-45-7
6.6	Permethrin trans	CAS#51877-74-8
6.7	Cyfluthrin	CAS#68369-37-5
6.8	Cypermethrin	CAS#52315-07-8
6.9	Fenvalerate	CAS#51630-58-1
6.10	Deltamethrin	CAS#52918-63-5
6.11	Resmethrin	CAS#10453-86-8

6.12 Acetone, nanograde or equivalent pesticide grade
6.13 Hexanes, nanograde or equivalent pesticide grade
6.14 Diethylether, nanograde or equivalent pesticide grade
6.15 Mason jars, pint size with lids
6.16 Magnesium sulfate, anhydrous
6.17 Whatman filter paper, #4, 15 cm
6.18 Funnels, short stem, 60°, 8 cm diameter
6.19 Copper powder, purified
6.20 Florisil SPE cartridge, 2 grams with 20 mL reservoir
6.21 Pipette, 1-mL
6.22 Test tube, 50 mL
6.23 Graduated conical tubes with glass stopper, 15-mL
6.24 Disposable Pasteur pipettes, and other laboratory ware as needed
6.24 Recommended analytical columns:

For ECD 5% (Phenyl)-methylpolysiloxane (HP-5MS or equivalent) fused silica column, 30 m x 0.25 mm id x 0.25 um film thickness.

DB608, (Specifically designed for the analysis of chlorinated pesticides and PCBs) 30 m x 0.25 mm id x 0.25 um film thickness

For MSD 5% (Phenyl)-methylpolysiloxane (HP-5MS or equivalent) fused silica column, 30 m x 0.25 mm id x 0.25 um film thickness.

7. Standards Preparation:

7.1 The individual pyrethroid stock standards of 1.0 mg/mL were obtained from the CDFA/CAC Standards Repository. The standards were diluted to 10 ng/µL with hexanes for identification purposes.

A combination standard of 10 µg/mL was prepared from individual mg/mL standards with acetone to be used for fortification. Another 10 µg/mL combination standard was prepared in hexanes and was diluted to the following concentrations: 0.005, 0.01, 0.025, 0.05, 0.1, 0.2 0.5 ng/µL in hexanes for instrument calibration.

7.2 Keep all standards in the designated refrigerator for storage.

7.3 The expiration date of each standard is six months from the preparation date.

8. Sample Preservation and Storage:

Store all samples waiting for extraction in a freezer. If samples are to be extracted the next day, they may be stored in the refrigerator. Sample extracts shall be stored in the refrigerator (32-40 °F).

9. Test Sample Preparation:

9.1 Background Preparation

The Department of Pesticide Regulation (DPR) provided the sediment for background to be used in method validation. The background sediment was provided in a 5 gal bucket. Excess water was decanted off before the sediment was mixed. The sediment was mixed well with a paddle attached to a drill and then passed through a Tyler equivalent #9 mesh sieve to remove debris. The sieved background was placed in quart size mason jars and stored in the refrigerator.

9.1.1 Blank

Remove background from refrigerator and allow it to come to room temperature. Mix well before weighing out 20 g of background. Proceed to step 9.2.2 of section 9.2.

9.1.2 Spike

Remove background from refrigerator and allow it to come to room temperature. Mix well before weighing out 20 g of background. Fortify at the level requested by client and mix well to ensure that the pesticides are well distributed. The spiked background was allowed to sit for 30 minutes before proceeding to step 9.2.2 of section 9.2.

9.1.3 Moistures

9.1.3.1 Thaw sediment sample and then decant any excess water from the sample. Thoroughly homogenized the sediment.

9.1.3.2 Weigh out a 15 – 20 g sub-sample into a pre-weighed aluminum weighing pan.

9.1.3.3 Dry pan with sediment for at least 6 hours in a ~ 105°C oven.

9.1.3.4 Reweigh sediment after cooling in a dessicator.

9.1.3.5 Report the wet and dry weights on Chain of Custody sample sheets.

9.2 Test Sample Extraction

9.2.1 Thaw sediment sample and then decant any excess water from the sample. Thoroughly homogenized the sediment and remove any debris (e.g., gravel, sticks). Weigh out a 20 $\pm$ 0.5 g sub-sample into a pint mason jar.

9.2.2 Add 5 g of copper powder to each sample and mix well. The copper powder eliminates the interferences in the ECD chromatograms caused by sulfur.

9.2.3 Place the Mason jar containing the sample on ice and add ~ 2 spatulas of anhydrous $MgSO_4$ and mix well. Keep adding $MgSO_4$ to the sample until it is dried (sandy condition).

9.2.4 Add 75 mL of 1:1 mixture of acetone/hexane to the mason jar, cover with foil and cap. Place on shaker and shake for 15 min at 185 rpm.

9.2.5 Decant the extract and filter through a piece of Whatman # 4 filter paper containing approximately 2 g anhydrous $MgSO_4$ into a 250 mL boiling flask. Repeat step 9.2.4 & 9.2.5 again, but this time transfer solvent and soil to the funnel and rinse with 1:1 acetone/hexane. The filtered extracts are combined.

9.2.6 Rotory evaporate to ~ 5 mL under vacuum at approximately 17-20 inch Hg in a water bath at 40° C.

9.2.7 Transfer the extract to a 15 mL graduated test tube. Rinse flask 3 times with approximately 2 mL of hexane and transfer each rinsate to the same test tube.

9.2.8 Place the test tube on nitrogen evaporator under a gentle stream of nitrogen with water bath set at 40° C and solvent-exchange with hexane. Bring to final volume of 2 mL.

Cleanup

9.2.8 Condition a 2 g florisil SPE cartridge with 10 mL of 15% diethylether followed by 20 mL hexane. Do not allow cartridges to go to dryness.

9.2.9 Carefully load the sample extract onto the conditioned florisil SPE cartridge. Rinse the tube that previously contained the extract twice with 2 mL hexane. Add rinses to florisil cartridge.

9.2.10 Elude the pesticides from the cartridge with 30 mL of 15% diethylether and collect in a 50 mL tube.

9.2.11 Evaporate the sample eluants to dryness under a gentle stream of nitrogen in a 40° C water bath.

9.2.12 Pipet 1mL of hexane into the test tube and vortex well. Remove 500 µL and place in an autosampler vial with insert, this vial is ready to be analyzed by GC-MSD. The final volume for MSD analysis is 1 mL.

9.2.13 Add 500 µL of hexane to the remaining contents in the tube and mix well. Vial contents of test tube into 2 autosampler vials with inserts to be analyzed by GC-ECD. The final volume for ECD analysis is 2 mL.

10. Instrument Calibration:

10.1 The calibration standard curve consists of a minimum of three levels. The recommended concentrations levels of standards are 0.005, 0.01, 0.025, 0.05, 0.1, 0.2, or 0.5 ng/µL.

10.2 The calibration curves for the ECD are obtained using piecewise. The MSD used linear regression with a correlation coefficient (r) equal to or greater than 0.995.

11. Analysis:

11.1 Injection Scheme

The instrument may need to be conditioned with a matrix blank or old sample before running the following sequence of Standard Curve, Hexane, Matrix Blank, Matrix Spike, Test Samples (maximum of 10 – 12) and Standard Curve.

11.2 GC-ECD Instrumentation

11.2.1 Analyze the pyrethroids extracts by a gas chromatograph equipped with dual electron capture detectors (ECD).

11.2.2 Recommended instrument parameters: Injector 225 °C; detector 300 °C Initial column temperature 150 °C, hold 2 min., ramp at 20 °C/min to final temperature 280 °C and hold for 15 min.; injection volume 2 µL. Flow rates of the Helium carrier gas were 3.8 mL/min. and 1.8 mL/min. for the HP-5MS and the DB-608 columns, respectively.

11.2.3 Due to matrix interferences in samples it may be necessary to use the modified column temperature program: 100 °C, hold 0 min., ramp at 10 °C/min to final temperature 230 °C and hold for 5 min, ramp at 2° C/min to final temperature of 280° C for 7 min.

11.3 GD-MSD Instrumentation

11.3.1 Analyze resmethrin by mass selective detector

11.3.2 Recommended instrument parameters: Injector 250 °C, msd transfer line heater 280 °C; initial column temperature 70 °C, hold 1 min., ramp at 22

°C/min. to final temperature of 280 °C and hold for 9 min.; injection volume 2 µL. Flow rate of Helium carrier gas was 1.0 mL/min.

Ions Selected for SIM Acquisition:

Resmethrin	123, 143, 171, 338 start time 6.00 min
Bifenthrin	165, 166, 181, 183 start time 11.00 min.
Fenpropathrin	97.0, 181, 265, 349 start time11.55 min.
λ Cyhalothrin epimer	181, 197, 208, 449 start time 11.95 min.
λ Cyhalothrin	181, 197, 208, 449 start time 11.95 min.
Permethrin cis	163, 165, 183, 184 start time 12.70 min.
Permethrin trans	163, 165, 183, 184 start time 12.70 min.
Cyfluthrin	163, 165, 206, 226 start time 13.20 min.
Cypermethrin	163, 165, 181, 209 start time 13.70 min.
Fenvalerate	167, 181, 225, 419 start time 14.80 min.
Deltamethrin	181, 209, 251, 253 start time 16.00 min.

12. Quality Control:

12.1 Method Detection Limits (MDL)

Method Detection Limit (MDL) refers to the lowest concentration of the analyte that a method can detect reliably. To determine the MDL, 7 sediment samples are spiked at 1.0 ppb and processed through the entire method along with a blank. The standard deviation derived from the spiked sample recoveries was used to calculate the MDL for each analyte using the following equation:

MDL = tS

Where t is the Student t test value for the 99% confidence level with n-1 degrees of freedom and S denotes the standard deviation obtained from n replicate analyses. For the n=7 replicates used to determine the MDL, t=3.143.

The results for the standard deviations and MDL are in Appendix 1.

12.2 Reporting Limit (RL)

Reporting limit (RL) refers to a level at which reliable quantitative results may be obtained. The MDL is used as a guide to determine the RL. The reporting limit

for all the pyrethriods is 1.0 ppb except for resmethrin, which is 1.5 ppb. This reporting limit was chosen after taking into account the matrix effect and various sample background that could be encountered.

12.3 Method Validation

The method validation consisted of five sample sets. Each set included four levels of fortification (1, 5, 20 and 400 ppb, except resmethrins lowest level is 3 ppb) and a method blank. All spikes and method blanks were processed through the entire analytical method. Recoveries for the pyrethroids are tabulated in Appendix 2.

12.4 Control Charts and Limits

Control charts were generated using the data from the method validation for each analyte. The upper and lower warning and control limits are set at $\pm$ 2 and 3 standard deviations of the % recovery, respectively, shown in Appendix 2.

12.5 Acceptance Criteria

12.5.1 Each set of samples will have a matrix blank and a spiked matrix sample.

12.5.2 The retention time should be within $\pm$ 2 per cent of that of the standards.

12.5.3 The recoveries of the matrix spikes shall be within the control limits.

12.5.4 The sample shall be diluted if results exceed the calibration curve.

13. Calculations:

Cyfluthrin, cypermethrin and fenvalerate are expressed as the sum of their isomers. Therefore, the total residues should be calculated using the sum of their peak responses.

Quantitation is based on external standard (ESTD) calculation using either the peak area or height. The ECD software uses a piecewise fit, with all levels weighted equally. The MSD uses linear regression fit, with all levels weighted equally. Alternatively, at chemist discretion, concentrations may be calculated using the response factor for the standard whose value is closest to the level in the sample.

$$ppb = \frac{(\text{sample peak area or ht}) \times (\text{std conc}) \times (\text{std vol. Injected}) \times (\text{final vol of sample})(1000)}{(\text{std.peak area or ht}) \times (\text{sample vol injected}) \times (\text{sample wt (g)})}$$

It may be necessary to dilute samples due to high background and the reporting limit would be multipled by the dilution factor.

14. Reporting Procedure:

14.1 The ECD HP-5ms is used as the primary column for reporting results for bifenthrin, fenpropathrin lambda cyhalothrin epimer, lambda cyhalothrin, fenpropathrin, permethrin cis and permethrin trans and cyfluthrin(peaks summed). Cypermethrin and fenvalerate/esfenvalerate(peaks summed) results were reported from the DB608 column due to cleaner chromatograms in the area where these peaks come out. Resmethrin results were reported from the MSD since it doesn't chromatogram well on the ECD. In some cases, however, certain analytes may have coeluting peaks associated with them and it may be necessary to use the DB608 column instead of the HP-5ms or visa versa.

14.2 Sample results are reported in accordance with the client's analytical laboratory specification sheets.

15. Discussion:

15.1 The fenvalerate standard is a ratio of approximately 60% fenvalerate and 40% esfenvalerate. The compound of interest is the esfenvalerate, but it was found from other studies that esfenvalerate in sample matrix degraded to fenvalerate over time. So the total of fenvalerate/esfenvalerate was calculated and reported. Deltamethrin was reported as deltamethrin/tralomethrin since deltamethrin and tralomethrin are indistinguishable by GC and GCMS methods.

15.2 Since the electron capture detector (ECD) is not truly and element specific detector, the MSD was used for further confirmation where concentrations were high enough. Further work is needed to confirm at the RL of 1ppb level for fenpropathrin, cyfluthrin, cypermethrin, fenvalerate/esfenvalerate and deltamethrin.

15.3 The sample matrix may require that the liner be changed more frequently and the column trimmed to maintain sensitivity.

15.4 This method was adapted from the methods listed in the references below.

16. References:

16.1 J. You, D.P. Weston, M. J. Lydy, *A Sonication Extraction Method for the Analysis of Prethroid, Organophosphate, and Organchlorine Pesticides from Sediment by Gas Chromatography with Electron-Capture Detection,* Archives Environmental Contamination and Toxicology 47, 141-147 (2004)

16.2 J. You, M. J. Lydy, *Evaluation of Desulfuration Methods for Pyrethroid, Organophosphate, and Organochloride Pesticides in Sediment with High Sulfur Content,* Archives Environmental Contamination and Toxicology 47, 148 -153 (2004)

Appendix 1

The determination of Method Detection Limit (MDL) and Reporting Limit (RL)

	Bifenthrin		Fenopropathrin		Lambda cyhalothrin epimer		Lambda cyhalothrin	
	ppb	%	ppb	%	ppb	%	ppb	%
blk sed	**n/d**		n/d		n/d		n/d	
spk1	**0.716**	71.6	**0.679**	67.9	**0.757**	75.7	**0.753**	75.3
spk2	**0.732**	73.2	**0.646**	64.6	**0.734**	73.4	**0.717**	71.7
spk 3	**0.732**	73.2	**0.697**	69.7	**0.756**	75.6	**0.770**	77.0
spk 4	**0.676**	67.6	**0.608**	60.8	**0.673**	67.3	**0.674**	67.4
spk 5	**0.670**	67.0	**0.625**	62.5	**0.716**	71.6	**0.713**	71.3
spk 6	**0.659**	65.9	**0.619**	61.9	**0.677**	67.7	**0.699**	69.9
spk 7	**0.653**	65.3	**0.612**	61.2	**0.678**	67.8	**0.674**	67.4
Std dev	**0.0345**		**0.0348**		**0.0373**		**0.0367**	
MDL	**0.1083**		**0.1094**		**0.1173**		**0.1154**	
RL	1.00ppb		1.00ppb		1.00ppb		1.00ppb	

	Permethrin cis		Permethrin trans		cyfluthrin		Cypermethrin	
	ppb	%	ppb	%	ppb	%	ppb	%
blk sed	**n/d**		n/d		n/d		n/d	
spk1	**0.632**	63.2	**0.628**		**0.903**	90.3	**0.765**	76.5
spk2	**0.594**	59.4	**0.588**		**0.735**	73.5	**0.703**	70.3
spk 3	**0.671**	67.1	**0.621**		**0.780**	78.0	**0.745**	74.5
spk 4	**0.684**	68.4	**0.698**		**0.738**	73.8	**0.718**	71.8
spk 5	**0.687**	68.7	**0.644**		**0.796**	79.6	**0.714**	71.4
spk 6	**0.694**	69.4	**0.702**		**0.783**	78.3	**0.657**	65.7
spk 7	**0.683**	68.3	**0.680**		**0.746**	74.6	**0.720**	72.0
Std dev	**0.0369**		**0.430**		**0.0582**		**0.0339**	
MDL	**0.1159**		**0.1352**		**0.183**		**0.107**	
RL	1.00ppb		1.00ppb		1.00ppb		1.00ppb	

Appendix 1(cont.)

	Fenvalerate/Esfenvalerate		Deltramethrin		Resmethrin	
	ppb	%	ppb	%	ppb	%
blk sed	**n/d**		n/d		n/d	
spk1	**0.719**	71.9	**0.702**	70.2	**2.07**	69.0
spk2	**0.662**	66.2	**0.662**	66.2	**2.30**	76.7
spk 3	**0.787**	78.7	**0.696**	69.6	**2.34**	78.0
spk 4	**0.679**	67.9	**0.657**	65.7	**2.49**	83.0
spk 5	**0.665**	66.5	**0.648**	64.8	**2.38**	79.3
spk 6	**0.663**	66.3	**0.658**	65.8	**1.90**	63.3
spk 7	**0.688**	68.8	**0.681**	68.1	**1.74**	58.0
Std dev	**0.0454**		**0.0210**		**0.2769**	
MDL	**0.143**		**0.0661**		**0.8702**	
RL	1.00ppb		1.00ppb		1.50ppb	

Appendix 2

Method Validation Data and Control Limits

	Bifenthrin	**Fenpropathrin**	**λ Cyhalothrin epimer**	**λ Cyhalothrin**	**Permethrin cis**	**Permethrin trans**
Spike Level (ppb)	Recovery(%)	Recovery(%)	Recovery(%)	Recovery(%)	Recovery(%)	Recovery(%)
1.0	77.1	66.5	74.0	72.8	70.7	62.4
	71.0	66.9	74.4	73.2	78.3	75.5
	77.3	70.3	77.7	77.8	77.1	62.5
	78.7	74.2	77.2	71.1	77.9	60.9
	74.6	70.7	76.4	76.8	81.7	78.0
5.0	68.2	62.0	68.2	71.0	67.0	67.0
	70.4	65.6	72.6	74.0	75.0	71.4
	74.4	66.9	73.8	76.2	72.2	69.0
	73.0	61.8	73.8	75.0	72.8	67.8
	72.2	66.6	74.4	73.2	71.4	68.8
20	84.0	77.5	84.5	85.0	82.0	83.0
	67.5	61.5	67.5	66.5	64.0	63.0
	80.5	74.0	80.5	80.5	78.5	75.0
	80.0	75.0	81.5	80.0	83.0	79.0
	79.5	75.5	81.5	82.5	82.5	80.0
400	85.8	85.8	89.0	90.5	87.3	88.0
	90.3	87.8	92.5	89.5	88.3	88.5
	86.8	80.8	85.5	87.3	85.5	84.3
	82.0	78.8	80.8	80.5	80.0	77.8
	89.8	86.0	90.5	88.8	88.0	86.3
Mean	78.2	72.7	78.8	78.6	78.2	74.4
SD	6.85	8.21	6.99	6.89	6.93	9.01
UCL	98.7	97.3	99.8	99.3	98.9	101
UWL	91.9	89.1	92.8	92.4	92.0	92.4
LWL	64.5	56.2	64.8	64.8	64.3	56.4
LCL	57.6	48.1	57.8	57.9	57.4	47.4

Appendix 2 (cont..)

	Cyfluthrin	**Cypermethrin**	**Fenvalerate/ Esfenvalerate**	**Deltamethrin**	**Resmethrin**
Spike Level (ppb)	Recovery(%)	Recovery(%)	Recovery(%)	Recovery(%)	Recovery(%)
1.0	78.9	81.5	78.1	72.5	69.3
	84.0	68.0	66.2	63.8	68.3
	94.6	78.7	79.3	74.9	67.3
	72.3	79.9	76.8	70.8	53.0
	92.6	76.3	75.9	69.0	67.0
5.0	70.0	62.8	71.8	63.2	69.6
	74.0	66.0	69.6	62.8	78.0
	80.4	69.0	75.4	68.2	69.6
	75.4	73.0	77.8	69.2	65.2
	105	63.9	68.2	62.6	61.6
20	79.5	70.5	81.0	80.0	67.0
	61.0	53.0	60.0	58.5	46.7
	74.0	66.5	72.0	71.0	70.5
	77.0	71.5	78.5	71.5	79.5
	76.5	79.0	81.0	80.0	66.0
400	87.5	70.0	76.8	86.0	65.3
	86.0	76.8	90.5	90.5	60.0
	85.0	73.5	82.3	83.3	62.5
	75.0	67.8	75.8	79.0	66.8
	85.8	81.5	92.0	96.8	74.3
Mean	80.7	71.5	76.5	73.7	66.4
SD	9.77	7.26	7.48	10.1	7.50
UCL	110	93.3	98.9	104	88.9
UWL	100	86.0	91.4	93.9	81.4
LWL	61.2	56.9	61.5	53.5	51.4
LCL	51.4	49.6	54.0	43.3	43.9

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Revision Log:

Date	What was Revised? Why?
12/01/05	Section 11.2.3 Due to matrix interferences in samples it may be necessary to use the modified column temperature program: 100 °C, hold 0 min., ramp at 10 °C/min to final temperature 230 °C and hold for 5 min, ramp at 2° C/min to final temperature of 280° C for 7 min. Added
12/01/05	Section 11.3.1 It may be necessary to dilute samples due to high background and the reporting limit would be multipled by the dilution factor.
	Section15.1 Deltamethrin was reported as deltamethrin/tralomethrin since deltamethrin and tralomethrin are indistinguishable by GC and GCMS methods.
2/28/07	Section Name Change, Environmental Analysis Section replaces Environmental Monitoring Section
	Personnel changes