

**Methods Development for the Analysis of Pyrethroid Pesticides in
Environmental Samples**

**FINAL REPORT FOR CALFED
Recipient Agreement No. ERP-02-P42**

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Project Background Information: With the phase-out of chlorpyrifos and diazinon, the use of pyrethroid insecticides has been increasing. Pyrethroids are very toxic and analytical methods need to be developed to measure them in water, sediment, colloids and biota at environmentally relevant concentrations. More than twenty pyrethroid insecticides are currently registered for use in California and are applied to a wide variety of crops including alfalfa, corn, rice, and tomatoes, as well as to most orchard crops. In addition, home use of pyrethroid insecticides is also increasing.

Objectives: The primary project goals are to develop, test, and validate methods for analysis of six or more pyrethroid insecticides in water, colloids, sediments, and biota. As part of the method development, each step of sample collection, processing, and analysis will be carefully tested, and modified if needed, to insure quantitative recovery and sufficient sensitivity. The analysis of pyrethroid insecticides in water and sediment will be a joint venture between three laboratories: U.S. Geological Survey's Organic Chemistry Laboratory in Sacramento (USGS), California Department of Fish and Game's Water Pollution Control Laboratory in Rancho Cordova (CDF-WPCL), and the California Department of Food and Agriculture's laboratory in Sacramento (CDFA) along with the California Department of Pesticide Regulation (CDPR). This approach gives us maximum expertise and access to a wide variety of instrumentation. The method will be validated by analysis of a limited number of environmental water samples by all three laboratories. The final result will be routine methods for water and sediment analyses that are operational in the three different laboratories and can be used by other laboratories with similar equipment. The CDFG laboratory will also develop biota methods while the USGS laboratory will focus on a method for colloids.

Results and Findings:

Development of Analytical Methods:

Pyrethroids are hydrophobic with log K_{ow} 's ranging from 4 to 7 (Laskowski, 2002, Physical and chemical properties of pyrethroids, Rev Environ Contam Toxicol 174: 49-170). They are expected to be associated with the sediment rather than in the water but it is important to develop methods for all environmental compartments to gain a better understanding of their overall fate and transport. Further information on the individual pyrethroids can be found on CDPR's environmental fate reviews at their website, <http://www.cdpr.ca.gov/docs/empm/pubs/envfate.htm>. Reviews have been completed for bifenthrin, cyfluthrin, cyhalothrin, cypermethrin, esfenvalerate and permethrin.

Routine analytical methods were developed for the analysis of currently-used pyrethroid insecticides (both home use and commercial use) in water, sediment colloids and biota with special attention to decreasing the detection limits in water to parts per trillion (ppt) levels or lower. The objective is to achieve reporting limits in water equal to or lower than the LC₅₀ of sensitive species such as *Daphnia magna* and *Ceriodaphnia dubia*. Reporting limits at the low parts per billion (ppb) level are the objective for sediment and biota analyses so that the methods will be useful for monitoring ambient concentrations of these pesticides. Toxicity levels of pyrethroids for select organisms in water and sediment are shown in Table 1.

Table 1. Toxicity values of pyrethroids 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	10 day LC ₅₀ (ng/g)	Organism
Bifenthrin	70	<i>Ceriodaphnia dubia</i>	4	<i>Americamysis bahia</i>	5	<i>Hyalella azteca</i>
Cyfluthrin	140	<i>Ceriodaphnia dubia</i>	2	<i>Americamysis bahia</i>	10	<i>Hyalella azteca</i>
λ-Cyhalothrin	300	<i>Ceriodaphnia dubia</i>	4	<i>Americamysis bahia</i>	5	<i>Hyalella azteca</i>
Cypermethrin	130	<i>Ceriodaphnia dubia</i>	5	<i>Americamysis bahia</i>	3-6	<i>Hyalella azteca</i>
Deltamethrin	37	<i>Daphnia magna</i>	17	<i>Americamysis bahia</i>		
Esfenvalerate	240	<i>Daphnia magna</i>	38	<i>Americamysis bahia</i>	15	<i>Hyalella azteca</i>
Permethrin	75	<i>Daphnia magna</i>	20	<i>Americamysis bahia</i>	110	<i>Hyalella azteca</i>

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

The initial proposal was to develop methods for bifenthrin, cyfluthrin, λ -cyhalothrin, cypermethrin, esfenvalerate and permethrin in all matrices. Additionally other pyrethroids were added (allethrin, deltamethrin, fenpropathrin, τ -fluvalinate, resmethrin, sumithrin (phenothrin), and tetramethrin) though not by all laboratories and not in all matrices. When possible standards of specific isomers were found. Some of the specific isomers purchased were: β -cyfluthrin, λ and γ -cyhalothrin, α,β,τ and ζ -cypermethrin, cis and trans-permethrin. A summary of pyrethroids analyzed by each laboratory is given in Table 2. Methods developed by each laboratory are summarized in Table 3 and each laboratories recoveries and MDLs summarized in Tables 4 through 6. All laboratories developed water and sediment methods, the USGS also developed a colloid method and CDFG developed a tissue method. Details for each method can be found in Appendix A. Detection limits are near the LC₅₀ values for both water (ppt levels) and sediment (ppb levels).

Table 2. Summary of individual pyrethroids analyzed by each laboratory (not necessarily in all matrices). The original six pyrethroids are listed first.

Pyrethroid	USGS	CDFG	CDFA
Bifenthrin	x	x	x
Cyfluthrin	x	x	x
Cyhalothrin	x	x	x
Esfenvalerate/Fenvalerate	x	x	x
Permethrin	x	x	x
Allethrin	x	x	
Deltamethrin	x	x	x
Fenpropathrin	x	x	x
τ -Fluvalinate	x		
Resmethrin	x	x	x
Sumithrin	x		
Tetramethrin	x	x	

Table 3. Summary of methods for pyrethroid analysis developed by each laboratory

Laboratory	Medium	Extraction Method	Volume	Analysis Methods	MDLs
USGS	Water	Filtered sample; SPE extraction with bottle rinse	1 L	GC-MS GC-MS/MS	2-6 ng/L 0.5-1 ng/L
	Sediment (bed and suspended)	MASE/ carbon and alumina/ GPC	5 g (dry weight)	GC-MS GC-MS/MS	1-2 ng/g 0.2-0.5 ng/g
	Colloids	SPME	18 mL	GC-MS	10-25 ng/L
CDFG	Water	Whole water; liquid:liquid extraction	1 L	GC-MS and GC-ECD	1-5 ng/L
	Sediment	ASE/GPC/Florisil	5 g (dry weight)	GC-MS and GC-ECD	1-4 ng/g
	Tissue	ASE/GPC/Florisil	10 g (fresh weight)	GC-MS and GC-ECD	1-5 ng/g
CDFA	Water	Whole water; liquid:liquid extraction	1 L	GC-MS and GC-ECD	1-8 ng/L
	Sediment	Shaking/Florisil	20 g (wet weight)	GC-MS and GC-ECD	0.1-0.9 ng/g

Table 4. USGS methods for the analysis of pyrethroids in water, sediment and colloids. MDL = method detection limit; LOD = limit of detection.

Pyrethroid	Water			Sediment			Colloids
	% Recovery (RSD)	MDL GC/MS (ng/L)	MDL GC/MS/MS (ng/L)	% Recovery (RSD)	MDL GC/MS (ng/g)	MDL GC/MS/MS (ng/g)	LOD (ng/L)
Allethrin	107 ± 7	6.0	1.2	72 ± 7	1.5	0.2	25
Bifenthrin	94 ± 6	4.7	0.7	77 ± 4	2.2	0.2	10
Cyfluthrin	89 ± 9	5.2	1.1	82 ± 6	2.0	0.5	25
λ-Cyhalothrin	85 ± 9	2.0	0.5	79 ± 9	2.4	0.2	10
Cypermethrin	85 ± 8	5.6	1.1	87 ± 8	2.6	0.4	25
Deltamethrin	96 ± 9	3.5	0.6	87 ± 8	2.5	0.2	10
Esfenvalerate	89 ± 8	3.9	0.5	83 ± 8	2.1	0.2	10
Fenpropathrin	88 ± 7	4.1	0.6	90 ± 6	2.1	0.2	10
τ-Fluvalinate	83 ± 9	5.3	0.7	99 ± 9	2.6	0.2	10
Permethrin	98 ± 8	3.4	0.6	93 ± 3	1.0	0.2	10
Resmethrin	92 ± 8	5.7	1.1	89 ± 6	1.9	0.5	25
Sumithrin	99 ± 8	5.1	1.0	101 ± 3	1.3	0.3	10
Tetramethrin	95 ± 5	2.9	0.5	83 ± 4	1.4	0.2	10

Table 5. CDFG methods for the analysis of pyrethroids in water, sediment and tissue. MDL = method detection limit.

Pyrethroid	Water		Sediment		Tissue
	% Recovery (RSD)	MDL (ng/L)	% Recovery (RSD)	MDL (ng/g)	MDL (ng/g)
Bifenthrin	88 ± 12	1.0	106 ± 3	0.4	0.7
Cyfluthrin	103 ± 13	1.7	108 ± 9	2.6	2.7
λ-Cyhalothrin	96 ± 13	0.8	104 ± 6	1.2	1.5
Cypermethrin	101 ± 9	1.1	109 ± 8	2.4	1.7
Deltamethrin	106 ± 24	1.6	62 ± 22	2.1	0.6
Esfenvalerate/Fenvalerate	101 ± 14	0.9	107 ± 6	0.8	1.7
Fenpropathrin	88 ± 9	2.0		2.0	1.4
Permethrin	102 ± 6	1.1	99 ± 15	3.8	2.2

Table 6. CDFA methods for the analysis of pyrethroids in water and sediments. MDL = method detection limit.

Pyrethroid	Water		Sediment	
	% Recovery (RSD)	MDL (ng/L)	% Recovery (RSD)	MDL (ng/g)
Bifenthrin	85 ± 8	1.8	78 ± 7	0.11
Cyfluthrin	100 ± 12	1.7	80 ± 10	0.18
λ-Cyhalothrin	96 ± 8	1.1	79 ± 7	0.12
Cypermethrin	100 ± 15	1.8	72 ± 7	0.11
Deltamethrin	96 ± 15	1.9	74 ± 10	0.07
Esfenvalerate/Fenvalerate	98 ± 14	1.8	77 ± 7	0.14
Fenpropathrin	99 ± 7	1.5	73 ± 8	0.11
cis-Permethrin	95 ± 9	3.5	78 ± 7	0.12
trans-Permethrin	98 ± 11	7.7	74 ± 8	0.14
Resmethrin	93 ± 12	3.8	66 ± 8	0.87

Pyrethroid Container Sorption

Pyrethroids in water can sorb to sampling containers (glass or plastic) with up to 50 percent associating with the container walls. This sorption is of analytical and toxicological importance; loss of pyrethroids during analysis or exposure studies will lead to skewed results. The amount of pyrethroid sorption that occurs depends on the composition of the container (glass, plastic) and the water (organic carbon content, suspended sediments).

Pyrethroid sorption with respect to analytical measurements is addressed for this report. Pyrethroid sorption to glass containers, which are the most common for pesticide samples is reversible. If the sample is transferred out of the glass container by pouring or after vigorous shaking, the amount of sorption is much lower than if the water is pumped out of the glass bottle at 10 mL/min (Figure 1). This is of importance for those laboratories that use solid-phase extraction (SPE) to analyze pyrethroids. A bottle rinse must be done after the water is pumped through the SPE cartridge to achieve a mass balance (Figure 2).

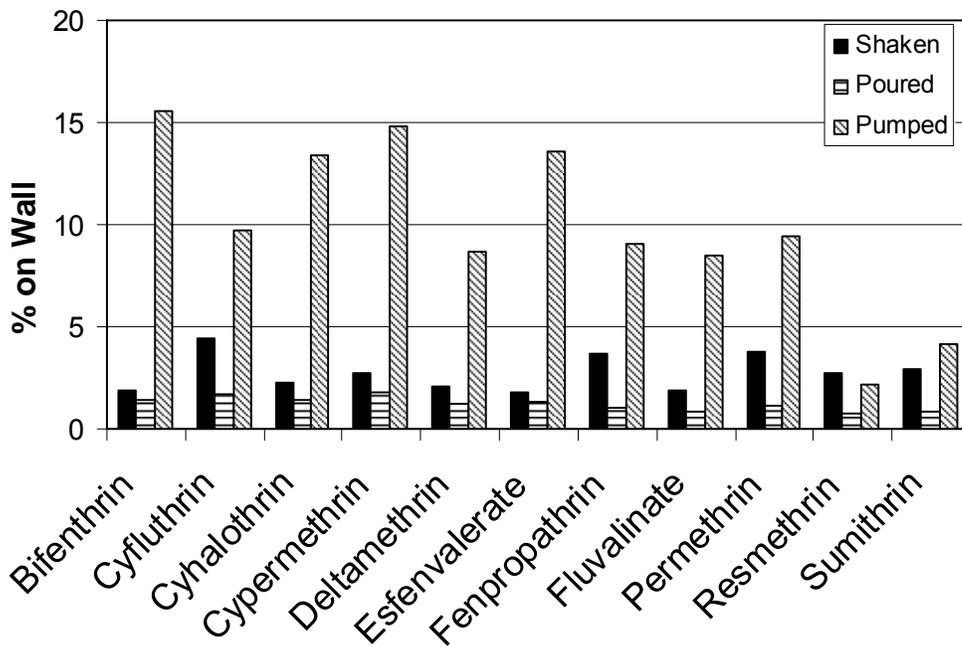


Figure 1. Percentage of pyrethroids left on the 1-L glass bottle was after shaking the bottle before pouring out the water, pouring out the water gently and pumping the water through an SPE cartridge (10 mL/min). Water used was from Colusa Basin Drain, filtered with 6 mg/L of DOC. Water was spiked at 400 ng/L and allowed to equilibrate for 24 hours.

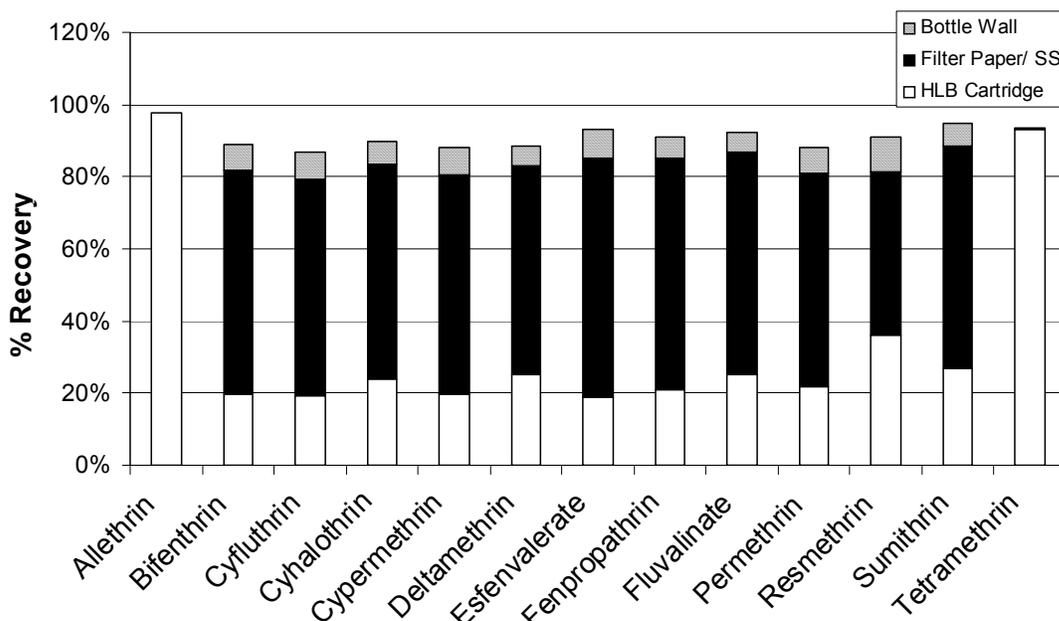


Figure 2. Mass balance of water sample spiked at 400 ng/L in a mixture of two natural waters (SSC = 13.9 mg/L and DOC = 5.0 mg/L). Sample sat for 3 days before extraction. The sample is divided into that which was sorbed to the suspended sediments, what was dissolved in the water and what sorbed to the bottle wall after pumping through the extraction cartridge.

Water and Sediment Method Validation

To validate the methods developed for measuring pyrethroids in water and sediment by CDFA, CDFG, and the USGS, samples were analyzed by each laboratory and the results were compared. Optimally natural water and sediment samples, with known detections of pyrethroids, would be used for the comparison. Through CDPR’s concurrent monitoring, sediment was collected near Salinas, California that had prior detects of pyrethroids. For water, there was no known area that had detects of multiple pyrethroids that could be used for this study. Instead, two natural waters were collected and then spiked with pyrethroids.

Water

Sample Preparation

All preparation of water samples was done by the USGS. Two waters were collected that would be spiked with pyrethroids. The first water was collected from the American River; this was used as “clean water” because of its low suspended sediment (< 10 mg/L) and dissolved organic carbon concentration (1 mg/L). To simulate the high-end of possible suspended sediments encountered in water sampling, sediment was taken from Colusa Basin Drain (by CDPR; previously dried) and was added to the water for a suspended sediment concentration of 500 mg/L. The SOP for creating the “sediment water” can be found at CDPR’s website; <http://www.cdpr.ca.gov/docs/empm/pubs/sops/QAQC009.pdf>. The second water was collected from Colusa Basin Drain and was not modified. The DOC concentration was 6.6 mg/L DOC and the suspended sediment concentration was 14 mg/L. Water was collected in 20-L stainless steel soda kegs. Each of the two waters was spiked with pyrethroids at two concentrations, 10 ng/L and 100 ng/L, in the soda kegs. Samples were pumped from the soda kegs (with continuous stirring) into 1 L amber glass bottles via a peristaltic pump at ~ 500 mL/min.

For each of the two waters, all laboratories received one bottle of non-spiked water, two bottles of water spiked at 10 ng/L and two bottles of water spiked at 100 ng/L, for a total of ten bottles. All laboratories also

received an aliquot of the pyrethroid spiking solution (20 ng/μL) in methanol. The bottles were labeled randomly and sent to each laboratory within four hours. Samples were extracted within 48 hours.

Results

None the laboratories detected any pyrethroids in the blank water samples. Concentrations of the spiking solution measured by all laboratories agreed within 10%. The results for the low-level (10 ng/L) pyrethroid spikes were inconclusive. One of the laboratories reporting limit is 15 ng/L and they were unable to measure the pyrethroids. The other two laboratories measured concentrations of 3-9 ng/L for each pyrethroid.

For the higher pyrethroid concentrations (100 ng/L) the American River water samples had fairly good agreement but concentrations measured were lower than expected (50-70% recovery). Results for the American River water are shown in Figure 3. This is most likely due to the high concentration of suspended sediments (500 mg/L) that complicated the extraction of the pyrethroids from the water. This level of suspended sediments represents that worst-case scenario that would be encountered. The Colusa Basin Drain (CBD) samples had better recovery (>70%) than those from the American River. The results for the CBD samples are shown in Figure 4. Additionally for the CBD samples most concentrations measured at each laboratory are within one standard deviation. The CBD water has a composition more similar to most waters sampled.

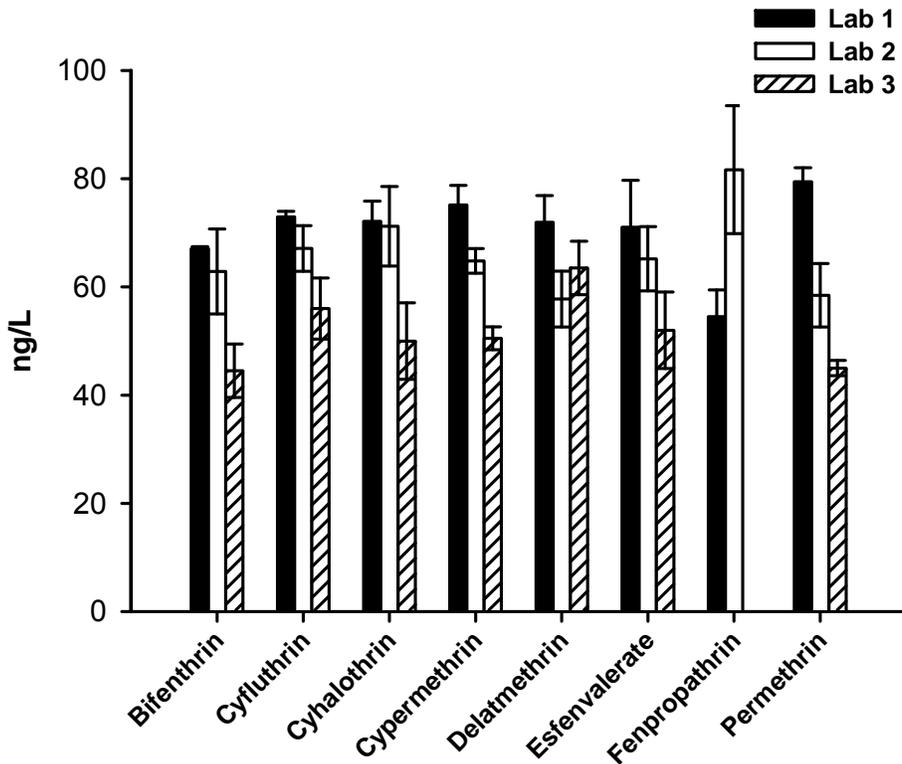


Figure 3. Results of pyrethroids spiked into American River water at 100 ng/L. American River water contained 500 mg/L of sediment. Error bars represent one standard deviation.

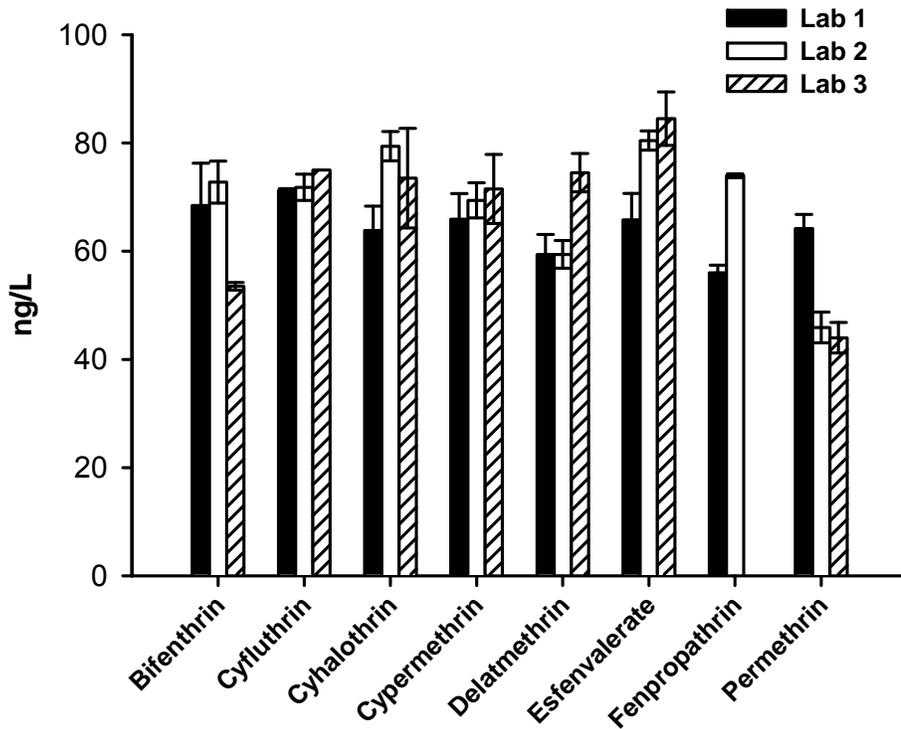


Figure 4. Results of pyrethroids spiked into Colusa Basin Drain water at 100 ng/L. Water had 14 mg/L of suspended sediment and 6 mg/L of DOC.

Sediment

Sample Collection

Sediment was collected from the Salinas area by CDP. The sediment was homogenized and all laboratories received 2 1-L glass jars of sediment. Extractions were completed within one month. USGS determined the organic carbon (OC) content of the sediment to be 3.2%.

Results

For the pyrethroids detected at concentrations greater than 10 ng/g, all three laboratories had similar results (Figure 5). Slight differences in concentrations could be due to extraction methods. Sonication has been shown to quantify 30% less than heated or pressurized extractions for organochlorine and organophosphorus pesticides on aged sediments (Eskilsson and Bjorkland, 2000, J. Chrom A., 902, 227-250).

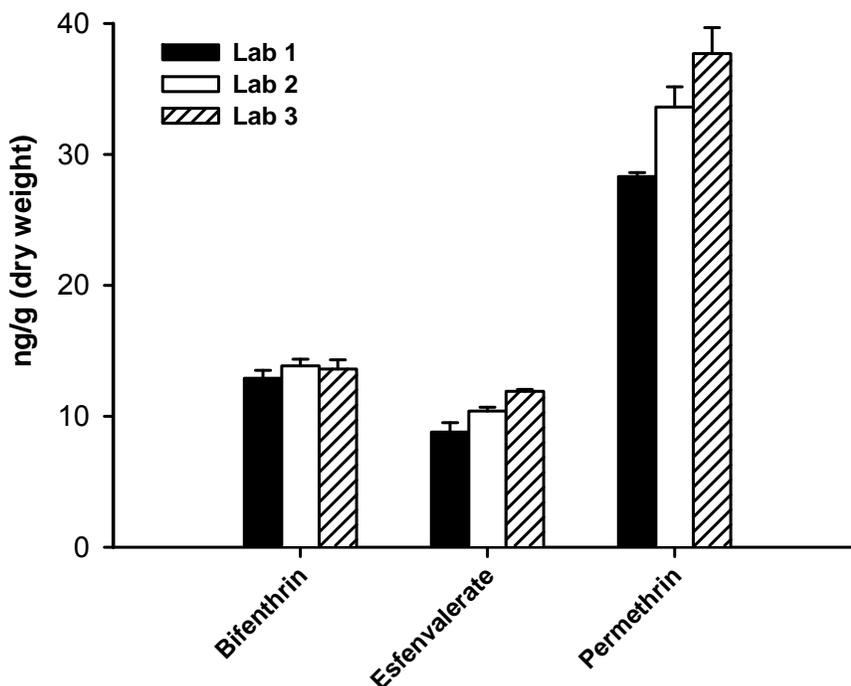


Figure 5. Results of pyrethroids detected in sediment collected near Salinas, California. Tissue Method

Standard and Sample Storage

Several tests were undertaken by the individual laboratories to test the stability of the pyrethroids in varying situations. The USGS tested the stability of standards and also of stored extracts. After six months of storage in a -20°C freezer it was found that the pyrethroid relative peak areas varied by no more than 10%.

CDFA undertook a study of the stability of pyrethroids in water samples that were refrigerated to 4°C . Recoveries of the pyrethroids were 87 to 100% at 0 days but decreased to 50 to 100% at 4 days and 50 to 90% at 7 days. To gain maximum recovery of pyrethroids in water samples they need to be analyzed within 24 hours of receipt. CDFA also explored adding a keeper solvent for the samples. The keeper solvent was 10 mL of hexane added to the 1 L water sample. With the keeper solvent recoveries were 95 to 100% at 0 days and 65 to 100% after 4 days with most compounds giving increased recoveries versus the absence of a keeper solvent. Additional details for this experiment can be found in Appendix A. The addition of a keeper works if liquid:liquid extractions are the method of analysis but will not work for SPE extraction.

The USGS tested the stability of pyrethroids that had been extracted on HLB and C8 cartridges. C8 cartridges were included because they are used in toxicity tests. The water can be passed through the cartridge, the cartridge is dried and stored frozen at -20°C . Cartridges were eluted each week up to four weeks. There was less than 15% change in the pyrethroid concentration after four weeks.

CDFA did storage tests on spiked sediment samples and found that they were stable over one month. The USGS has extracted natural samples over one year with less than 10% change in the concentration measured.

**METHODS DEVELOPMENT FOR THE ANALYSIS OF PYRETHROID
PESTICIDES IN ENVIRONMENTAL SAMPLES**

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Appendix A: USGS, CDFG and CDFA Pyrethroid Methods

USGS SOP for the determination of Pyrethroids in Water

I. Introduction

To determine the toxicity, fate and transport of pyrethroid insecticides it is necessary to be able to analyze these compounds in water

II. Scope and Application

This method measures pyrethroids in the dissolved phase. The method covers the following pyrethroids: allethrin, bifenthrin, cyfluthrin, λ -cyhalothrin, cypermethrin, deltamethrin, esfenvalerate, fenpropathrin, τ -fluvalinate, permethrin and sumithrin (phenothrin) and tetramethrin.

III. Summary of Method

Method based on Hladik et al., 2007 uses Oasis HLB solid phase extraction (SPE) cartridges to extract pyrethroids from the water. The extract is concentrated and quantification of individual pyrethroids is done by gas chromatography/mass spectrometry. The filter paper can also be extracted with organic solvent and analyzed for pyrethroids.

IV. Samples Collection, Preservation and Holding Times

Samples should be collected in amber glass jars (1 L). Sample should be stored in a refrigerator (4 °C) until analysis; samples should be analyzed within three days of collection and preferably within 24 hours.

V. Interferences

Method interferences may be caused by contaminants in the reagent water, reagents, glassware, and other sample processing apparatus that bias analyte response. All glassware should be heated at 450 °C for 8 hours prior to using. For sample extraction components that cannot be heated, they must be washed, rinsed with DI water, dried and rinsed with dichloromethane and acetone (pesticide grade or better).

VI. Materials/Equipment Required

A. Safety

It is recommended for laboratory personnel to wear safety glasses, gloves, and laboratory coats as protection from samples and reagents.

B. Apparatus

1. Glass fiber filters (0.7 μ m)
2. Large volume water pump
3. Water Oasis HLB SPE cartridges (6 mL, 500 mg)

4. SPE vacuum manifold
5. Pump with Teflon tubing and power supply
6. Organomation N-evap and 12 mL concentrator tubes
7. Branson 5200 sonicator
8. Zymark Turbovap II
9. Varian Saturn gas chromatograph/mass spectrometer (GC/MS) with DB-5ms column (30 m × 0.25 mm × 0.25 μm)
10. Pre-cleaned pipettes, syringes, flasks, glass funnels

C. Reagents

1. Calibration Curve – 0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 1.0, 2.5, 5 ng/μL pyrethroids in ethyl acetate
2. Calibration Check – 0.5 ng/μL pyrethroids in ethyl acetate
3. Matrix Spike – 2 ng/μL pyrethroids in ethyl acetate
4. Surrogate Spike – 2 ng/μL phenoxy-¹³C₆-*cis*-permethrin in ethyl acetate
5. Internal Standard – 40 ng/μL of d₁₀-acenaphthene, d₁₀-phenanthrene, and d₁₀-pyrene in ethyl acetate

VII. Procedure

Filtration:

1. Label a 1L glass bottle with sample information such as date, location, time and type of sample.
2. Clean the filtering apparatus, without a fiber filter in place, by pumping 100 mL of methanol into methanol waste bottle, followed by 500 mL DI organic free water into plastic beaker (to be poured down sink).
3. Open filter setup and place fiber filter carefully in place using forceps (DO NOT TOUCH FILTER WITH YOUR HANDS). Close filter and tighten clamp with hand. Open pressure valve at the top of the filter apparatus. Start pumping sample through filter allowing the first 50 mL or so to go into plastic beaker or down the drain. The pressure valve at the top of the filter apparatus is left open until water starts squirting out, then it is closed immediately.
4. Collect ~ 1L of filtered sample in the labeled bottle.
5. When finished, open filter setup, remove used filter with forceps and place in aluminum foil and freeze for later analysis or discard.
6. Using a squirt bottle filled with organic free water, squirt off any filter residues and close filter apparatus.
7. Clean the filter setup, without fiber filter in place, by pumping through 100 to 200 mL methanol into methanol waste bottle, followed by 500 to 800 mL organic free water into plastic beaker or down drain.
8. Continue on to next sample or if finished, wrap ends of tubing in foil and crimp.

Extraction:

1. Fill out a Laboratory Analysis form for each filtered sample.
2. Measure 1000 mL (1 liter) of sample with a clean one-liter graduated cylinder. If the volume of sample is less than 1000 mL, measure the volume accurately. To clean the graduated cylinder, rinse with organic-free water (to remove any remaining sediment particles, rinse sparingly with methanol, and finish with three rinses of organic-free water. ALWAYS record sample volume on laboratory analysis form.
3. Remove water surrogate, from freezer allowing it to warm to room temperature. This step is very important to measure the correct volume (you can also warm the vial quickly in your hands, if needed). Slowly pull up 100 μ L of the surrogate with a microsyringe and then gently wipe the outer surface of the needle. Add the surrogate to the sample by placing the tip under the water surface and slowly expelling the surrogate into the sample. Shake the sample well. If sample is a matrix spike, add 200 μ L of matrix spike (if more than one spike, add 200 μ L of each matrix spike) to sample using a microsyringe in the manner described above and shake well.
4. Before use, the solid phase extraction (SPE) HLB cartridges must be clean. Using manifold, allow two column volumes of ethyl acetate to gravity drip through cartridge followed by two column volumes of methanol and then by one column volume of organic free water making sure that some water is left above the frit (a few cm of water above the frit).
5. Label cartridge and cover label with clear tape (use printed out label if possible). The label should contain the sample location, date, and analysis type. If it is a QA sample such as a blank or a matrix, please label as such.
6. Extraction pumps should be clean prior to use (if not previously cleaned) by pumping through 25-50 mL of methanol (methanol waste collected in methanol waste container) followed by 200-500 mL of organic-free water.
7. Attach the cartridge to the pump and begin to pump sample through cartridge at a flow rate of 10 mL per minute. Measure the flow rate with a small graduated cylinder several times during the extraction. Just a tip, make sure cartridge is full of organic-free water before attaching to pump. And also make sure there is organic-free water in the tubing rather than air before the pumping is started so as not to push air through the cartridge. Keep an eye (or ear) on the extraction as sometimes the tubing pops off the SPE cartridge. If the cartridge becomes clogged and the sample will not pump through, you will need to use another cartridge for the sample.
8. Once the extraction is complete, the cartridges are dried by placing the cartridge of the manifold and vacuum pumping any remaining water. The cartridges are then further dried with CO₂ using the manifold and timer. After drying for an hour, the cartridges are either immediately eluted or put in a ziplock bag with a label (date or study or other pertinent information) and placed in the freezer for storage (up to one month).
9. Include any important comments, such as added surrogate twice, lost some sample when extracting etc. on the laboratory analysis form. **THERE IS NO SUCH THING AS UNNECESSARY INFORMATION!**
10. Remember to clean the pumps by pumping through 25-50 mL of methanol followed by 200-500 mL of organic free water.

11. Bottle rinse: add sodium sulfate to the empty glass bottle to remove any residual water. Add approximately 4 mL of methylene chloride, cap the bottle and gently roll the solvent around the bottle. Empty the solvent into a concentrator tube and repeat methylene chloride rinse two more times. Using the N-evap, reduce the methylene chloride fraction to ~0.5-1 mL.
12. To elute the sample put the cartridge on the manifold (be sure the cartridge is at room temperature if it had been stored in the freezer). Elute with 12 mL of ethyl acetate, collect the ethyl acetate in a concentrator tube. Using the N-evap gently blow the sample down to a few mL, add the bottle rinse and then blow down the sample to 200 μ L. Add 40 μ L of internal standard and transfer sample to GCMS vial.

Filter Paper:

1. Remove the filter paper from the freezer and dry at room temperature overnight, in the dark (cover lightly with aluminum foil).
2. Cut up filter and place in a 250 mL Erlenmeyer flask
3. Extract with 2×75 mL of 1:1 methylene chloride:acetone in the sonicator for 30 minutes
4. Filter the solvent through a GF/F, 0.7 μ m filter
5. Reduce extract using the turbovap to 0.5 mL.
6. Exchange the extract into ethyl acetate and further reduced to 200 μ L with the N-evap
7. Add 40 μ L of internal standard

GC/MS:

1. Inject sample extracts (1 μ L) onto GC/MS
2. GC/MS Parameters: injector is set at 275 $^{\circ}$ C, and the trap, manifold and transfer line temperatures are set at 220, 80, and 280 $^{\circ}$ C respectively. GC oven program is: 80 $^{\circ}$ C (hold 0.5 min), ramp to 300 $^{\circ}$ C at 10 $^{\circ}$ C/min and hold for 5 min.
3. Selected ion storage (SIS) windows are set according to Table 1.
4. For GC/MS/MS parameters are set according to Table 2.
5. Quantification is done using Varian Workstation software

Table 1. Retention times, selected ion storage (SIS) levels and quantitation ions for pyrethroids analyzed by GC/MS (ion-trap).

Compound	Retention Time (min)	SIS Storage Levels	Quantitation Ions (m/z)
Allethrin	14.99	90-450	123
Resmethrin	17.91	95-146, 163-179	143+171
Bifenthrin	18.36	93-100, 119-143, 158-186, 195-201, 262-269	181
Tetramethrin	18.42		164
Fenpropathrin	18.52		181+265
Sumithrin	18.91		123+183
Cyhalothrin	19.32		181
Permethrin	20.22	89-95, 149-170, 178-201, 224-229	183
Cyfluthrin	20.74		127+163+199
Cypermethrin	21.06		127+163+181
Fluvalinate	22.01	123-129, 149-156, 165-184, 223-228, 248-257	250
Esfenvalerate	22.02		225
Deltamethrin	22.54		253

Table 2. Parameters for pyrethroids analyzed by GC/MS/MS.

Compound	Parent Ion (m/z)	Excitation Storage Level (m/z)	Excitation Amplitude (V)	Quantitation Ions (m/z)
Allethrin	123	54	41	67+81+95
Bifenthrin	181	79.7	67	153+165+166
Cyfluthrin	163	71.7	58	91+127+167
λ -Cyhalothrin	181	79.7	87	151+152+153
Cypermethrin	181	79.7	86	151+152+153
Deltamethrin	253	111.5	62	172+174
Esfenvalerate	225	99.1	82	119+142+169
Fenpropathrin	265	116.8	85	172+210+236
τ -Fluvalinate	250	110.2	100	180+194+200
Permethrin	183	80.5	74	153+165+168
Resmethrin	143	62.9	53	128+141
Sumithrin	183	80.5	75	153+168+181
Tetramethrin	164	72.1	61	77+91+107

VIII. Calculation

Concentration of each pesticide is calculated using the calibration curve with the Varian Workstation software. The software will give a mass (ng) for the 1 μ L injection (ng/ μ L). This

mass will have to be multiplied by the sample volume (200 µL) and divided by the dry weight of the original sample (g).

IX. Quality Assurance/Quality Control

1. Calibration curve – a calibration curve consisting of a minimum of 5 points should be processed with the batch. The R^2 must be > 0.995 .
2. Blank – a minimum of one blank should be processed with a batch of 20.
3. Matrix Spike (MS) – one MS and one MS duplicate should be processed with a batch of 20. Compound recovery should fall between 70-130% of the standard concentration (minus any detected in the sample).
4. Replicate – one sample duplicate should be process for every 20 samples.
5. Continuing Calibration Verification (CCV) should be analyzed every 6 samples. The CCV should be near the midpoint of the calibration curve.
6. Limits of detection are determined by taking the lowest calibration standard and back calculating to the concentration found in the sample
7. Method detection limits (MDLs) are determined by spiking 10-20 µL into 1L of Sacramento River water (concentration of 20-40 ng/L) and the sample carried through the entire procedure. The MDL was calculated according to the EPA procedure (USEPA, 1992) using the following equation:

$$0.025 \text{ ng/}\mu\text{L std} \times 200 \mu\text{L of extract} \div 5 \text{ g of sample} = 1 \text{ ng/g}$$

$$\text{MDL} = S \times t(n-1, 1-\alpha = 0.99)$$

where:

S = standard deviation of replicate samples

n = number of replicates

t = value of Student's t statistic at n-1 degrees of freedom and 99 percent confidence level

Table 3. Limits of detection (LOD), percent recovery and relative standard deviation (RSD) and method detection limits (MDL) for pyrethroids in water.

Compound	LOD (ng/L)	% Recovery (RSD)	MDL GC/MS (ng/L)	MDL GC/MS/MS (ng/L)
Allethrin	5	107 ± 7	6.0	1.2
Bifenthrin	2	94 ± 6	4.7	0.7
Cyfluthrin	5	89 ± 9	5.2	1.1
λ-Cyhalothrin	2	85 ± 9	2.0	0.5
Cypermethrin	5	85 ± 8	5.6	1.1
Deltamethrin	2	96 ± 9	3.5	0.6
Esfenvalerate	2	89 ± 8	3.9	0.5
Fenpropathrin	2	88 ± 7	4.1	0.6
τ-fluvalinate	2	83 ± 9	5.3	0.7
Permethrin	2	98 ± 8	3.4	0.6
Resmethrin	5	92 ± 8	5.7	1.1
Sumithrin	2	99 ± 8	5.1	1.0
Tetramethrin	2	95 ± 5	2.9	0.5

X. Waster Collection and Disposal

Used extraction cartridges and filter paper can be placed in the trash. Solvent waste should be placed in an organic waste container and disposed as hazardous waste.

XI. References

Hladik, M.L., Smalling, K.L., Kuivila, K.M., 2007, A multi-residue method for the analysis of pesticides and pesticide degradates in water using Oasis HLB solid-phase extraction and gas chromatography-ion trap mass spectrometry. *Bulletin of Environmental Contamination and Toxicology*, In press.

U.S. Environmental Protection Agency, 1992, Definition and procedure for the determination of the method detection limit—revision 1.11, Code of Federal Regulations 40, Protection of the Environment, CFR Part 136, Appendix B, p. 565-567.

USGS SOP for the determination of Pyrethroids in Sediment

I. Introduction

To determine the toxicity, fate and transport of pyrethroid insecticides it is necessary to be able to analyze these highly hydrophobic compounds in sediment

II. Scope and Application

This method (Smalling et al, 2005) measures pyrethroids associated with sediments. The method covers the following pyrethroids: bifenthrin, cyfluthrin, λ -cyhalothrin, cypermethrin, deltamethrin, esfenvalerate, fenpropathrin, τ -fluvalinate, permethrin and sumithrin (phenothrin).

III. Summary of Method

Microwaves assisted extraction (MAE) is used to extract pyrethroids from the sediment. The extract is concentrated and the matrix is removed from the extract by passing the sample through carbon and alumina. Quantification of individual pyrethroids is done by gas chromatography/mass spectrometry.

IV. Samples Collection, Preservation and Holding Times

Samples should be collected in amber glass jars (500 mL). Minimum sample size for pyrethroid extraction (and accompanying percent moisture) is 50 g. Sample should be stored frozen (-20 °C) until analysis; samples may be held up to 12 months at this temperature.

V. Interferences

Method interferences may be caused by contaminants in the reagent water, reagents, glassware, and other sample processing apparatus that bias analyte response. All glassware should be heated at 450 °C for 8 hours prior to using. For sample extraction components that cannot be heated, they must be washed, rinsed with DI water, dried and rinsed with dichloromethane and acetone (pesticide grade or better).

VI. Materials/Equipment Required

A. Safety

It is recommended for laboratory personnel to wear safety glasses, gloves, and laboratory coats as protection from samples and reagents.

B. Apparatus

1. Balance capable of weighing 0.01 g
2. CEM MSP 100 MAE-, including pre-cleaned extraction vessels
3. Zymark TurboVapII, including pre-cleaned tubes (200 to 1 mL)
4. Organomation N-evap and 12 mL concentrator tubes

5. SPE vacuum manifold
6. Sep-Pak Alumina A cartridges
7. Carboprep carbon cartridges (500 mg)
8. Gel Permeation Chromatography (GPC) clean-up with PL-gel column (50 Å, 300 × 7.5 mm)
9. Varian Saturn gas chromatograph/mass spectrometer (GC/MS) with DB-5ms column (30 m × 0.25 mm × 0.25 μm)
10. Pre-cleaned pipettes, syringes, flasks, glass funnels

C. Reagents

1. Calibration Curve - 0.025, 0.05, 0.1, 0.25, 0.5, 1.0, 2.5, 5 ng/μL pyrethroids in ethyl acetate
2. Calibration Check – 0.5 ng/μL pyrethroids in ethyl acetate
3. Matrix Spike – 2 ng/μL pyrethroids in ethyl acetate
4. Surrogate Spike – 2 ng/μL phenoxy-¹³C₆-*cis*-permethrin in ethyl acetate
5. Internal Standard – 40 ng/μL of d₁₀-acenaphthene, d₁₀-phenanthrene, and d₁₀-pyrene in ethyl acetate

VII. Procedure

Microwave Extraction:

1. Turn on MAE and allow to warm-up for 20-30 min.
2. Rinse all MAE vessels and caps with dichloromethane (DCM) and acetone before use.
3. Start with wet sediment. If frozen, thaw overnight in refrigerator.
4. Weigh ~5.0 g dry weight (calculate how much wet weight equivalent) into pre-cleaned, labeled MAE vessels.
5. Spike 100 μl of 2 ng/μL surrogate spike [+ 100 μL matrix spike (2 ng/μL) in some cases] onto sediment surface.
6. Set % moisture of sediment to 50 % by adding the appropriate amount of water. If over 50 % moisture do not add water to the sediment.
(50 - % moisture of sediment/100) * wet weight of sediment sample
7. Add 30 mL 90:10 DCM: Methanol to each sample vessel and insert into sleeve.
8. Cap MAE vessels tightly and place into MAE. Make sure the vessels are spaced evenly throughout the tray. Load method (Sediment: 75 % power; Time: 20 minutes; TAP: 15 min; Temp: 120 °C). Let cool for about 20 min (pressure needs to be <5 psi before removing MAE vessels).
9. Setup glass funnels with Na₂SO₄. After extraction pour samples over Na₂SO₄ to dry and into an appropriate collection vessel. Rinse Na₂SO₄ 2 x's and discard.
10. Repeat microwave extraction on sediment remaining in microwave vessels using 30 ml DCM:Methanol (9:1). Do not add more water to the sediment unless it looks dry. When completed and cooled, pour supernatant through fresh Na₂SO₄ and into the same collection vessel.
11. Transfer extract to Turbo-vap tube, place in Turbo-vap and reduce to < 0.5 mL.

Matrix Removal:

1. Stack a carbon SPE cartridge on top of an alumina cartridge.
2. Rinse cartridges with 3 column volumes of DCM. **IMPORTANT:** do not allow to cartridges to go dry.
3. After the cartridges are washed place graduated test tubes in the rack. Add sample directly to top of carbon cartridge and rinse graduated test tube with DCM.
4. Elute the cartridges first with 10 mL of DCM at ~ 1-2 drops/second.
5. Reduce to 0.5 mL with N-evap. Add 0.5 mL ethyl acetate, then reduce again to < 0.5 mL.

GPC:

1. Turn on pump and UV/Vis lamp and allow them to warm up for 30 minutes (flow rate = 1.0 mL/min; pressure 160-180 psi).
2. Turn on plotter.
3. To determine collection window inject 500 μ L of pyrethroid standard (2 ng/ μ L). Immediately following injection, hit the 'runtime' key on the plotter and start the stop watch.
4. Once the absorbance starts to increase hit the runtime key on the plotter a second time. At the end of the window when the absorbance drops back to ~0.02 hit the runtime key. Subtract the time at the beginning of the window and at the end of the window from the initial time at injection. This will give you a collection window. In order to make sure you get all compounds give the window a 30 second buffer on each side. Usual collection window ranges from 7-15 minutes.
5. After determining the collection window, inject the entire sample into the GPC and make sure to note the injection volume on the lab form. Immediately after the sample is injected start the stopwatch and hit the runtime key on the plotter.
6. Place the graduated test tube in the collection beaker and remove the waste hose at the appropriate time. At the end of the window replace the waste hose and allow solvent to pump through the GPC for another 40 minutes (sulfur should come out between ~ 20 minutes after the end of your collection window). Make sure to turn off the plotter after sample collection.
7. Reduce sample to 0.2 mL, add 40 μ L of deuterated IS and transfer to GC/MS vial.

GC/MS:

1. Inject sample extracts (1 μ L) onto GC/MS
2. GC/MS Parameters: injector is set at 275 $^{\circ}$ C, and the trap, manifold and transfer line temperatures are set at 220, 80, and 280 $^{\circ}$ C respectively. GC oven program is: 80 $^{\circ}$ C (hold 0.5 min), ramp to 300 $^{\circ}$ C at 10 $^{\circ}$ C/min and hold for 5 min.
3. Selected ion storage (SIS) windows are set according to Table 1.
4. For GC/MS/MS parameters are set according to Table 2.

5. Quantification is done using Varian Workstation software

Table 1. Retention times, selected ion storage (SIS) levels and quantitation ions for pyrethroids analyzed by GC/MS (ion-trap).

Compound	Retention Time (min)	SIS Storage Levels	Quantitation Ions (m/z)
Resmethrin	17.91	95-146, 163-179	143+171
Bifenthrin	18.36	93-100, 119-143, 158-186, 195-201, 262-269	181
Fenpropathrin	18.52		181+265
Sumithrin	18.91		123+183
Cyhalothrin	19.32		181
Permethrin	20.22	89-95, 149-170, 178-201, 224-229	183
Cyfluthrin	20.74		127+163+199
Cypermethrin	21.06		127+163+181
Fluvalinate	22.01	123-129, 149-156, 165-184, 223-228, 248-257	250
Esfenvalerate	22.02		225
Deltamethrin	22.54		253

Table 2. Parameters for pyrethroids analyzed by GC/MS/MS.

Compound	Parent Ion (m/z)	Excitation Storage Level (m/z)	Excitation Amplitude (V)	Quantitation Ions (m/z)
Allethrin	123	54	41	67+81+95
Bifenthrin	181	79.7	67	153+165+166
Cyfluthrin	163	71.7	58	91+127+167
λ -Cyhalothrin	181	79.7	87	151+152+153
Cypermethrin	181	79.7	86	151+152+153
Deltamethrin	253	111.5	62	172+174
Esfenvalerate	225	99.1	82	119+142+169
Fenpropathrin	265	116.8	85	172+210+236
τ -Fluvalinate	250	110.2	100	180+194+200
Permethrin	183	80.5	74	153+165+168
Resmethrin	143	62.9	53	128+141
Sumithrin	183	80.5	75	153+168+181
Tetramethrin	164	72.1	61	77+91+107

VIII. Calculation

Concentration of each pesticide is calculated using the calibration curve with the Varian Workstation software. The software will give a mass (ng) for the 1 μ L injection (ng/ μ L). This

mass will have to be multiplied by the sample volume (200 μL) and divided by the dry weight of the original sample (g).

IX. Quality Assurance/Quality Control

1. Calibration curve – a calibration curve consisting of a minimum of 5 points should be processed with the batch. The R^2 must be > 0.995 .
2. Blank – a minimum of one blank should be processed with a batch of 20.
3. Matrix Spike (MS) – one MS and one MS duplicate should be processed with a batch of 20. Compound recovery should fall between 70-130% of the standard concentration (minus any detected in the sample).
4. Replicate – one sample duplicate should be process for every 20 samples.
5. Continuing Calibration Verification (CCV) should be analyzed every 6 samples. The CCV should be near the midpoint of the calibration curve.
6. Limits of detection are determined by taking the lowest calibration standard and back calculating to the concentration found in the sample
7. Method detection limits (MDLs) are determined by spiking 50 ng into 5 g of Cache Creek sediment (concentration of 10 ng/g) and the sample carried through the entire procedure. The MDL was calculated according to the EPA procedure (USEPA, 1992) using the following equation:

$$0.025 \text{ ng}/\mu\text{L std} \times 200 \mu\text{L of extract} \div 5 \text{ g of sample} = 1 \text{ ng/g}$$

$$\text{MDL} = S \times t(n-1, 1-\alpha = 0.99)$$

where:

S = standard deviation of replicate samples

n = number of replicates

t = value of Student's t statistic at n-1 degrees of freedom and 99 percent confidence level

Table 3 Limits of detection (LOD), percent recovery and relative standard deviation (RSD) and method detection limits (MDL) for pyrethroids in sediment. All are based on dry weight.

Compound	LOD (ng/g)	% Recovery (RSD)	MDL GCMS (ng/g)	MDL GC/MS/MS (ng/g)
Allethrin	1	72 \pm 7	1.5	0.2
Bifenthrin	1	77 \pm 4	2.2	0.2
Cyfluthrin	2	82 \pm 6	2.0	0.5
λ -Cyhalothrin	1	79 \pm 9	2.4	0.2
Cypermethrin	2	87 \pm 8	2.6	0.4
Deltamethrin	1	87 \pm 8	2.5	0.2
Esfenvalerate	1	83 \pm 8	2.1	0.2
Fenpropathrin	1	90 \pm 6	2.1	0.2
τ -Fluvalinate	1	99 \pm 9	2.6	0.2
Permethrin	1	93 \pm 3	1.0	0.2
Resmethrin	1	89 \pm 6	1.9	0.5
Sumithrin	1	101 \pm 3	1.3	0.3
Tetramethrin	1	83 \pm 4	1.4	0.2

X. Waster Collection and Disposal

Extracted sediment should be dried in a hood, once dry it can be thrown in the trash. Solvent waste should be placed in an organic waste container and disposed as hazardous waste.

XI. References

Smalling, K.L., Orlando, J.L., and Kuivila, K.M., 2005, Analysis of Pesticides in Surface Water and Sediment from Yolo Bypass, California, 2004-2005: U.S. Geological Survey Scientific Investigations Report 2005-5220, 20 p.

U.S. Environmental Protection Agency, 1992, Definition and procedure for the determination of the method detection limit—revision 1.11, Code of Federal Regulations 40, Protection of the Environment, CFR Part 136, Appendix B, p. 565-567.

USGS SOP for the determination of Pyrethroids Associated with Colloids

I. Introduction

To determine the toxicity, fate and toxicity of pyrethroid insecticides it is necessary to be able to analyze these compounds associated with colloids.

II. Scope and Application

This method measures pyrethroids associated with colloids. The method covers the following pyrethroids: allethrin, bifenthrin, cyfluthrin, λ -cyhalothrin, cypermethrin, deltamethrin, esfenvalerate, fenpropathrin, τ -fluvalinate, permethrin and sumithrin (phenothrin) and tetramethrin.

III. Summary of Method

Method uses solid-phase microextraction (SPME) to determine total pyrethroids associated with colloidal material. Prior to analysis the colloids are concentrated from water samples with the Ultrafilter. The pyrethroids are quantified by gas chromatography/mass spectrometry.

IV. Samples Collection, Preservation and Holding Times

Colloids samples should be collected from the Ultrafilter in amber glass jars (1 L). Sample should be stored in a refrigerator (4 °C) until analysis; samples should be analyzed within three days of collection and preferably within 24 hours.

V. Interferences

Method interferences may be caused by contaminants in the reagent water, reagents, glassware, and other sample processing apparatus that bias analyte response. All glassware should be heated at 450 °C for 8 hours prior to using. For sample extraction components that cannot be heated, they must be washed, rinsed with DI water, dried and rinsed with dichloromethane and acetone (pesticide grade or better).

VI. Materials/Equipment Required

A. Safety

It is recommended for laboratory personnel to wear safety glasses, gloves, and laboratory coats as protection from samples and reagents.

B. Apparatus

1. SPME Fiber- 7 μ m, 1 cm PDMS
2. Varian Saturn gas chromatograph/mass spectrometer (GC/MS) with DB-5ms column (30 m \times 0.25 mm \times 0.25 μ m)
3. 20 mL sample vials with crimp cap tops

4. Pre-cleaned pipettes, syringes, flasks, glass funnels

C. Reagents

1. Calibration Curve – water samples (18 mL) with pyrethroids spiked at 10, 25, 50, 100, 250, 500, 1000 ng/L
2. Calibration Check/Matrix Spike – 250 ng/L pyrethroids in 18 mL water sample
3. Surrogate Spike – 250 ng/L phenoxy-¹³C₆-*cis*-permethrin in 18 mL water sample

VII. Procedure

Sample Preparation:

1. Label a 20 mL sample bottle with sample information such as date, location, time and type of sample.
2. Place 18 mL of sample into sample bottle.
3. Spike with surrogate for a concentration of 250 ng/L in 18 mL
4. Place crimp cap on vial.

SPME Extraction and GC/MS:

1. Pre-condition SPME fiber at 320 °C for 1 hour
2. Expose SPME fiber to sample for 30 min at 90 °C at 500 rpm
3. Desorb sample in injector at 275 °C for 3 min.
4. GC/MS Parameters: injector is set at 275 °C, and the trap, manifold and transfer line temperatures are set at 220, 80, and 280 °C respectively. GC oven program is: 80 °C (hold 0.5 min), ramp to 300 °C at 10 °C/min and hold for 5 min.
5. Bakeout of fiber is done at 275 °C for 10 min.
6. Selected ion storage (SIS) windows are set according to Table 1.
7. Quantification is done using Varian Workstation software

Table 1. Retention times, selected ion storage (SIS) levels and quantitation ions for pyrethroids analyzed by GC/MS (ion-trap).

Compound	Retention Time (min)	SIS Storage Levels	Quantitation Ions (m/z)
Allethrin	14.99	90-450	123
Resmethrin	17.91	95-146, 163-179	143+171
Bifenthrin	18.36	93-100, 119-143, 158-186, 195-201, 262-269	181
Tetramethrin	18.42		164
Fenpropathrin	18.52		181+265
Sumithrin	18.91		123+183
Cyhalothrin	19.32		181
Permethrin	20.22	89-95, 149-170, 178-201, 224-229	183
Cyfluthrin	20.74		127+163+199
Cypermethrin	21.06		127+163+181
Fluvalinate	22.01	123-129, 149-156, 165-184, 223-228, 248-257	250
Esfenvalerate	22.02		225
Deltamethrin	22.54		253

VIII. Calculation

Concentration of each pesticide is calculated using the calibration curve with the Varian Workstation software. The software will give a concentration (ng/L) for the 18 mL sample.

IX. Quality Assurance/Quality Control

1. Calibration curve – a calibration curve consisting of a minimum of 5 points should be processed with the batch. The R^2 must be > 0.995 .
2. Blank – a minimum of one blank should be processed with a batch of 20.
3. Matrix Spike (MS) – one MS and one MS duplicate should be processed with a batch of 20. Compound recovery should fall between 70-130% of the standard concentration (minus any detected in the sample).
4. Replicate – one sample duplicate should be process for every 20 samples.
5. Continuing Calibration Verification (CCV) should be analyzed every 6 samples. The CCV should be near the midpoint of the calibration curve.
6. Limits of detection are determined by taking the lowest calibration standard that responds on the GC/MS

Table 2. Limits of detection (LOD) for pyrethroids associated with colloids.

Compound	LOD (ng/L)
Allethrin	25
Bifenthrin	10
Cyfluthrin	25
λ -Cyhalothrin	10
Cypermethrin	25
Deltamethrin	10
Esfenvalerate	10
Fenpropathrin	10
τ -fluvalinate	10
Permethrin	10
Resmethrin	25
Sumithrin	10
Tetramethrin	10

X. Waster Collection and Disposal

Used extraction cartridges and filter paper can be placed in the trash. Solvent waste should be placed in an organic waste container and disposed as hazardous waste.

Determination of Synthetic Pyrethroids in Water Samples

1.0 Scope and Application

- 1.1 This method describes the sample preparation and quantitative analysis of trace level synthetic pyrethroids in surface, municipal and wastewater using liquid-liquid extraction and high resolution gas chromatography with electron capture detector (GC/ECD) and gas chromatography with mass spectrometer and ion trap detector (GC/MS-ITD) for confirmation. The following target analytes can be determined by this method:

<u>Analyte</u>	<u>CAS No.</u>
Bifenthrin	82657-04-3
Cyfluthrin	68359-37-5
Cypermethrin	52315-07-8
Deltamethrin	52918-63-5
Esfenvalerate/ Fenvalerate	66230-04-4/ 51630-58-1
Fenpropathrin	64257-84-7
Lambda-cyhalothrin	91465-08-6
Permethrin	52645-53-1

- 1.2 The method detection limit (MDL), reporting limits (RL) in ppb and average percent recoveries for each analyte is listed in Table 1. The method detection limits were calculated using USEPA procedures found in Title 40 Code of Federal Regulations Part 136 (40CFR 136, Appendix B, revision 1.11) as shown in Appendix A. The actual MDL may differ from those listed, depending upon the nature of interferences in the sample matrix.
- 1.3 This method can also be extended to include other pyrethroids. Table 2 lists these analytes, with their estimated method detection limits, estimated reporting limits and initial validation results in American River water (average percent recoveries and standard deviations for three replicates).
- 1.4 If possible, unknowns in the sample will be qualitatively confirmed for compound identification by gas chromatography equipped with an ion trap mass spectrometer detector (GC/MS-ITD).

Table 1. Synthetic pyrethroids analyzed by GC/ECD, their Minimum Detection Limits (MDL), Reporting Limits (RL) in ppb and average percent recoveries in water.

Target Analytes	MDL ($\mu\text{g/l}$)	RL ($\mu\text{g/l}$)	Average % Recoveries
Bifenthrin	0.001	0.002	88.1
Cyfluthrin	0.002	0.004	103
Cypermethrin	0.002	0.004	101
Deltamethrin	0.002	0.004	106
Es/Fenvalerate	0.001	0.002	101
Fenpropathrin	0.002	0.004	88.4
Lambda-cyhalothrin	0.001	0.002	96.2
Permethrin	0.003	0.005	102

Table 2. Additional pyrethroid compounds, estimated MDL and RL, average percent recoveries and standard deviations in American River water; spike level 20 ng/L.

Pyrethroids by GC/ECD	Est. MDL ($\mu\text{g/L}$)	Est. RL ($\mu\text{g/L}$)	AVERAGE % Recovery	STD DEV
Allethrin	0.001	0.003	105	5.85
Flucythrinate*	0.001	0.002	85.3	2.65
Phenothrin	0.025	0.050	90.5	4.36
Prallethrin	0.001	0.002	91.4	4.09
Tetramethin	0.002	0.005	114	2.00
Tralomethrin	0.002	0.005	50.3	6.40

*Flucythrinate co-elutes with cypermethrin on both columns on GC/ECD.

2.0 Summary of Method

- 2.1 A measured volume of sample (1000 ml) is extracted with methylene chloride (DCM) using a separatory funnel. The DCM extract is dried with sodium sulfate, evaporated using Kuderna-Danish (K-D) and solvent exchanged into petroleum ether. The extract is concentrated with micro-snyder (micro K-D) apparatus to approximately 1 ml and adjusted to 2.0 ml with iso-octane. The extracts are analyzed by gas chromatography using conditions which permit the separation and measurement of the target analytes in the extracts by GC/ECD.

- 2.2 Interferences in analyses may be encountered in very dirty samples and cleanup may be needed to aid in the elimination or reduction of these interferences. Florisil column cleanup or Gel Permeation Chromatography (GPC) procedures will be followed.

3.0 Interferences

- 3.1 Solvents, reagents, glassware, and other sample processing hardware may cause GC artifacts and/or elevated baselines, resulting in the misinterpretation of chromatograms. All materials should be demonstrated to be free from interferences under the conditions of the analysis by running method blanks initially and with each sample lot. Specific selection of reagents and purification of solvents by distillation in all-glass systems are required. High-purity distilled-in-glass solvents are commercially available.

An effective way of cleaning laboratory glassware is by rinsing with polar and non-polar solvents before use. The cleaning procedure used must be tested by analyzing procedural blanks prior to analyzing samples.

- 3.2 Phthalates are common laboratory contaminants that are used widely as plasticizers. Sources of phthalate contamination include plastic lab-ware, plastic tubing, plastic gloves, plastic coated glassware clamps, and have been found as a contaminant in Na_2SO_4 .

Polytetrafluoroethylene (PTFE) can be used instead of polypropylene or polyethylene to minimize this potential source of contamination. However, use of PTFE lab-ware will not necessarily preclude all phthalate contamination. Na_2SO_4 can be solvent rinsed to eliminate contaminants.

- 3.3 Matrix interferences may be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences will vary considerably from source to source. A Florisil or GPC cleanup procedure can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the MDL listed in Table 1.

4.0 Safety

- 4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound must be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in

the chemical analysis. Additional references to laboratory safety are available and have been identified for the information of the analyst.

5.0 Apparatus and Laboratory Supplies

- 5.1 Separatory funnel. 2000-ml, with TFE-fluorocarbon stopcock, ground glass or TEF stopper.
- 5.2 Automatic shaker designed to fit 2 liter separatory funnels with rpm and timer controls.
- 5.3 Beakers. Borosilicate glass, 400 mL
- 5.4 Glass wool. Pyrex - solvent washed prior to use.
- 5.5 Kuderna-Danish (K-D) Apparatus.
 - 5.5.1 Concentrator tube. 15 mL, graduate (Kontes K0570012-0500, or equivalent). A ground stopper, 19/22 joint, is used to prevent evaporation of extracts.
 - 5.5.2 Evaporation flask. 500 mL (Kontes K-570050-0500, or equivalent), attached to concentrator tube with blue clamp (Kontes K-662750-0012).
 - 5.5.3 Snyder column. Three ball (Kontes K-503000-0121, or equivalent).
 - 5.5.4 Micro-Snyder column. Alltech 9058 or equivalent.
 - 5.5.5 Boiling chips. Hengar granules, high purity amphoteric alundum - extracted with acetone and petroleum ether. Note that boiling chips can be a significant source of contamination if not properly cleaned.
- 5.6 Water bath. Blue M, 115 V, thermostatically controlled with stainless steel cover to fit K-D apparatus, installed in a fume hood.
- 5.7 GC vials. GC autosampler vials, borosilicate glass, 2 mL with PTFE-lined screw cap.
- 5.8 Analytical balance. Capable of weighing 0.1 mg.
- 5.9 Drying oven.
- 5.10 Disposable Pasteur Pipettes. 2 mL, rinsed with solvents before use.
- 5.11 Glass filter funnel. Fluted, 75 mm or larger.

- 5.12 Graduated cylinder. 1000 ml, 250 mL and 100 mL.
- 5.13 Culture tubes. 13 x 100 mm with PTFE lined screw cap.
- 5.14 Analytical systems
- 5.14.1 Gas chromatograph. Agilent 6890 equipped with dual ^{63}Ni micro electron capture detectors (ECD) with EPC, split-splitless injector, a 7683 autosampler and dual capillary columns (J&W Scientific) connected to a single injection port using a 5 meter pre-column with a "Y" press fit connector. Section 9 describes the acquisition and analysis procedures while Table 3 lists the operating parameters.
- 5.14.2 Gas chromatograph. Varian 3800, equipped with a Varian Saturn model 2000 or 4000 Ion Trap Mass Spectrometer, split-splitless injector, LEAP Model CTC A200SE autosampler and a 30 meter capillary column (J&W Scientific). Table 4 lists the operating parameters.
- 5.14.3 Data System. Hewlett-Packard, to collect and record GC/ECD data, generates reports, computes and records response factors for multi-level calibrations. Data system should be capable of calibrating a method using a minimum of 5 concentrations of analytical standards and calculating in external standard mode.

Table 3 Operating parameters for Agilent 6890 GC/ECD

Gases: Carrier: Helium, 1 mL/min
 Makeup: Nitrogen, 1 mL/min

Columns

DB-5, 60 m x 0.25 mm I.D. x 0.25 μm film thickness

DB-17-MS, 60 m x 0.25 mm I.D. x 0.25 μm film thickness

Mode: Constant flow

Initial flow: 1.9 mL/min

Inlet Isothermal: 240 $^{\circ}\text{C}$

Oven Program 1- Multi-residue

Initial temperature: 80 $^{\circ}\text{C}$, initial time: 1.00 min

Ramp 1: 15.0 deg/min, final temp 210 $^{\circ}\text{C}$, hold time 10.00 min

Ramp 2: 2.0 deg/min, final temp 290 $^{\circ}\text{C}$, hold time 14.00 min

Runtime: 73.67 min

Oven Program 2-Pyrethroids only

Initial temperature: 130 °C, hold for 1.00 min

Ramp 1: 20 deg/min, final temp 240°C, hold time 7.00 min

Ramp 2: 2 deg/min, final temp 290°C, hold time 16.50 min

Runtime: 55.00 min

Detectors (⁶³Ni μECD)

Temperature: 310 °C

Combined Flow: 31.0 mL/min (column + make-up flow)

Injection Volume: 3 μL

Table 4 Operating parameters for Varian Saturn GC/MS-Iron Trap Detector

Carrier gas: Helium

Columns: DB5MS, 30 m x 0.25 mm I.D. x 0.25 μm film thickness

Varian 1078 Inlet: Isothermal @ 260 °C

Injection Volume: 2 μL

Oven:

Initial temperature: 80 °C

Initial time: 1.00 min

Ramp 1: 15.0 deg/min, final temp 210 °C, hold time 10.00 min

Ramp 2: 2.0 deg/min, final temp 280 °C, hold time 0.50 min

MS-MS Operating Conditions

Trap Temperature: 240 °C

Manifold Temperature: 80 °C

Transferline Temperature: 280 °C

Ionization mode: EI Auto

Ion preparation: MS/MS

Scan time: 3μ scan/sec

Emission current: 50 μAmps

Waveform type: Non-resonant

Filament delay: 5 minute

6.0 Reagents, materials, gases and standards

- 6.1 Reagent water is defined as water in which an interferent is not observed at method detection limit of each parameter of interest. Deionized (DI) water was used for method validation and as method blank.
- 6.2 Petroleum ether (PE), acetone, methylene chloride (DCM), diethyl ether, isooctane. Pesticide residue quality or equivalent.
- 6.3 Sodium sulfate. Anhydrous granular reagent grade, rinsed with PE prior to use.
- 6.4 Nitrogen. Ultra-pure (99.99999%) for GC/ECD
- 6.5 Helium. Ultra-pure (99.99999%) for GC/ECD
- 6.6 Stock standards. Individual stock standards (100 µg/ml) are purchased as certified solutions from AccuStandard (New Haven, CT), ChemService (West Chester, PA) and Ultra Scientific (North Kingstown, RI).

7. CALIBRATION

- 7.1 Establish gas chromatographic operating parameters equivalent to those indicated in Table 3. The gas chromatographic system may be calibrated using either the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).
- 7.2 External standard calibration procedure
 - 7.2.1 For each parameter of interest, prepare calibration standards at a minimum of three concentration levels by adding accurately measured volumes of one or more stock standards to a volumetric flask and diluting to volume with hexane or other suitable solvent. One of the external standards should be representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates or should define the working range of the detector.
 - 7.2.2 Using injections of 1 to 5 µL of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each parameter. Alternatively, the ratio of the response to the mass injected, defined as the calibration factor (CF), may be calculated for each parameter at each standard concentration. If the relative

standard deviation of the calibration factor is less than 10% over the working range, the average calibration factor can be used in place of a calibration curve.

- 7.2.3 The working calibration curve or calibration factor must be verified on each working shift by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that parameter.
- 7.3 Internal standard calibration procedure: To use this approach, the analyst must select one or more internal standards similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Due to these limitations, no internal standard applicable to all samples can be suggested.
- 7.3.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask. To each calibration standard, add a known constant amount of one or more internal standards, and dilute to volume with hexane or other suitable solvent. One of the standards should be representative of a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates, or should define the working range of the detector.
- 7.3.2 Using injections of 1 to 5 μL of each calibration standard, tabulate the peak height or area responses against the concentration for each compound and internal standard. Calculate response factors (RF) for each compound as follows:

Equation 1

$$RF = (A_s) (C_{is}) / (A_{is}) (C_s)$$

where

A_s = Response for the parameter to be measured

A_{is} = Response for the internal standard

C_{is} = Concentration of the internal standard, in $\mu\text{g/L}$

C_s = Concentration of the parameter to be measured, in $\mu\text{g/L}$

If the RF value over the working range is constant, less than 10% relative standard deviation, the RF can be assumed to be invariant and the average RF may be used for calculations. Alternatively, the results may be used to plot a calibration curve of response ratios, A_s / A_{is} against RF.

- 7.3.3 The working calibration curve or RF must be verified on each working shift by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.
- 7.4 The cleanup procedure in Section 11 utilizes Florisil chromatography. Florisil from different batches or sources may vary in adsorptive capacity. To standardize the amount of Florisil which is used, the use of the lauric acid value is suggested. This procedure determines the adsorption from hexane solution of lauric acid, in milligrams, per gram of Florisil. The amount of Florisil to be used for each column is calculated by dividing this factor into 110 and multiplying by 20 g.
- 7.5 Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interference from the reagents.

8. QUALITY CONTROL

- 8.1 Each laboratory using this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated.
- 8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.
- 8.1.2 In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications to the method are made, the analyst is required to repeat the procedure in Section 8.2.
- 8.1.3 The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This

procedure is described in Section 8.4.

8.2 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.

8.2.1 Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in acetone, 1000 times more concentrated than the selected concentrations.

8.2.2 Using a pipette, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.

8.2.3 Calculate the average percent recovery (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.

8.2.4 Using the data from Table 3, estimate the recovery and single-operator precision expected for the method, and compare these results to the values calculated in Section 8.2.3. If the data are not comparable, review potential problem areas and repeat the test.

8.3 The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.

8.3.1 Calculate upper and lower control limits for method performance as follows:

$$\text{Upper Control Limit (UCL)} = R + 3s$$

$$\text{Lower Control Limit (LCL)} = R - 3s$$

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts that are useful in observing trends in performance.

8.3.2 The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as $R \pm s$. The accuracy statement should be developed by the analysis of four

aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s. Alternatively, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly.

- 8.4 The laboratory is required to collect in duplicate a portion of their samples to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one spiked sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular parameter does not fall within the control limits for method performance, the results reported for that parameter in all samples processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.
- 8.5 Before processing any samples, the analyst must demonstrate through the analysis of a 1-L aliquot of reagent water that all glassware and reagent interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank must be processed as a safeguard against laboratory contamination.
- 8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be analyzed to monitor the precision of the sampling technique. When doubt exists over the identification of a peak on the chromatogram, confirmatory techniques such as gas chromatography with a dissimilar column, specific element detector, or mass spectrometer must be used. Whenever possible, the laboratory should perform analysis of quality control materials and participate in relevant performance evaluation studies.

9.0 Sample Collection, Preservation, and Storage

- 9.1 Samples are collected in one liter amber glass bottles and iced or refrigerated at 4 °C from time of collection until extraction.
- 9.2 All samples must be extracted within 7 days and completely analyzed within 40 days of extraction.

10.0 Sample Extraction

- 10.1 Remove water samples from refrigerator and allow samples to reach room

temperature prior to extraction. Transfer contents to a pre-cleaned 2-liter separatory funnel. Immediately add 1.0 ml of the 20 ppb Dibromooctafluorobiphenyl (DBOB, CAS# 10386-84-2) surrogate solution to every sample. For laboratory control spike (LCS) and matrix spikes (MS/MSD) also add 1.0 ml of 20 ppb pyrethroid spiking solution.

- 10.2 Add 60 ml of methylene chloride (DCM) to the empty bottle, replace the cap and rinse the bottle. Pour the DCM into the separatory funnel and repeat with another 60 mL aliquot of DCM. Extract the sample by shaking the funnel for 5 minutes on the auto-shaker with periodic venting to release excess pressure. Allow organic layer to separate from the water phase for a minimum of 10 minutes. Collect the methylene chloride extract in a 400 ml beaker.
- 10.3 Add a second 120 ml volume of methylene chloride to the separatory funnel and repeat the extraction procedure a second time, combining the extracts in the beaker.
- 10.4 Set up and label pre-cleaned K-D flasks with concentrator tubes and attached with a blue clamp on ring stands in the fume hood. Add 0.5 ml iso-octane as “keeper” and a solvent rinsed micro-boiling chip to each K-D concentrator tube. Place a filter funnel containing a plug of pre-cleaned glass wool in the bottom of the funnel and place the funnel in the top of the K-D flask. Add about two inches of solvent rinsed sodium sulfate to the funnel.
- 10.5 Pour the combined extracts from the beaker through sodium sulfate into the K-D flask. Rinse the beaker with about 10 mL of DCM and add this rinse to the sodium sulfate. Repeat with another 10 mL DCM rinse. Rinse the sodium sulfate with an additional portion of DCM (~10-20 mL).
- 10.6 Place a Snyder column on the K-D flask, clamp with a green clamp and place the flask on the hot water bath set at 78-82 °C. Evaporate solvent on the hot water bath. When the apparent volume of solvent in the concentrator tube is 5-10 mL, add 20-30 mL of petroleum ether through the top of the Snyder column. Repeat this procedure when the apparent volume is again at 5-10 mL. When the reflux line falls below the top of the Snyder column, the K-D apparatus should be removed from the hot water bath. Dry the outer KD apparatus with a Kimwipe to prevent condensation water from entering the concentrator tube. Upon cooling, remove the concentrator tube from the K-D apparatus.
- 10.7 Place a clean micro-Snyder column on the concentrator tube with a blue clamp, add a new micro boiling chip and place in a 400 mL beaker containing water heated to approximately 78 °C on a hot plate. If the solvent does not begin to boil, remove the tube from the bath immediately,

allow it to cool slightly, add a new micro boiling stone to prevent it from bumping and place it back in the bath.

- 10.8 When the solvent has been evaporated to 0.5-1 mL remove the tube from the bath and allow it to cool in a test tube rack. Dry the outer KD apparatus with a Kimwipe to prevent condensation water from entering the concentrator tube. Remove the micro-Snyder column and add iso-octane to the concentrator tube to reach a final volume of 2.0 mL. Mix the tube contents by tapping the bottom of the tube causing a vortex which will rinse the sides of the tube. A Vortex Genie mixer may be used for this step.
- 10.9 Transfer the solution from the concentrator tube to a culture tube and cap with a Teflon™ faced cap. Place extracts in a refrigerator for storage until analysis or cleanup, if necessary.
- 10.10 When ready for analysis, transfer extract to labeled GC vials and cap.

11.0 Cleanup Procedure

- 11.1 Cleanup of dirty samples may be necessary due to interferences in the analysis of baseline or co-elution with target analytes of the sample extract. Follow the in-house SOP for Florisil® column or GPC method, as needed.

12.0 Analytical Procedure

- 12.1 The final extract will be analyzed on an Agilent 6890 GC/ECD. Chromatographic conditions for operating the Agilent 6890 GC/ECD are found in Table 3.
- 12.2 GC acquisition
 - 12.2.1 Analyze a Pesticide Degradation Check Solution (Ultra Scientific) at the beginning and end of each run to ensure GC performance.
 - 12.2.2 Pour several isooctanes into GC vials using the same lot as used for samples with each GC run.
 - 12.2.3 Pour standard curves into GC vials using 0.5, 1.0, 2.0, 5.0, 10, 20 and 50 ppb Pyrethroid Std in isooctane. Pour extra vials of a mid-level concentration for use as CCV (to be analyzed every 20 samples or less).

12.2.4 Create sequence file and sequence table on computer. Use the WPCL login number for "Data Subdirectory" and "Save As" sequence name.

12.2.5 Acquire data and recap each vial daily to preserve sample integrity.

12.3 Analysis

12.3.1 Recalibrate pyrethroid curves and analyze samples in external standard mode. Add a printed chromatogram and report for each standard and sample to folder.

13.0 References

U.S. Environmental Protection Agency, Office of Water, EPA 821-R-92-002, April 1992, Methods For The Determination of Nonconventional Pesticides In Municipal And Industrial Wastewater, p. 771. Method 1660, *The Determination of Pyrethrins and Pyrethroids in Municipal and Industrial Wastewater*.

U.S. Environmental Protection Agency, Office of Solid Waste, SW-846 On-Line, Method 3510C, *Separatory Funnel Liquid-Liquid Extraction*, Revision 3, December 1996,
<http://www.epa.gov/epaoswer/hazwaste/test/pdfs/3510c.pdf>, [06-27-06]

U.S. Environmental Protection Agency, Office of Solid Waste, SW-846 On-Line, Draft Update IVB, Method 8081B, *Organochlorine Pesticides by Gas Chromatography*, Revision 2, November 2000
http://www.epa.gov/epaoswer/hazwaste/test/pdfs/8081b_ivb.pdf [06-27-06]

SOP Section Approval: _____

Date: _____

SOP Final Approval: _____

Date: _____

SOP QA Officer Approval: _____

Date: _____

APPENDIX A

METHOD DETECTION LIMIT VALIDATION FOR PYRETHROIDS IN WATER

Pyrethroid Pesticides	Spike Amount Expected (ng/L)	Average Amount Recovered (ng/L)	Average Percent Recovery	Variance Amount Recovered (ng/L)	Standard Deviation Amount Recovered	MDL=t*Stdev (ng/L)
Bifenthrin	2.00	1.76	88.1	0.097	0.312	0.98
Cyfluthrin-1	4.00	3.87	96.6	0.178	0.422	1.33
Cyfluthrin-2	4.00	4.10	102	0.152	0.390	1.23
Cyfluthrin-3	4.00	4.30	108	0.116	0.341	1.07
Cyfluthrin-4	4.00	4.24	106	1.07	1.03	3.25
Cypermethrin-1	4.00	4.48	112	0.166	0.408	1.28
Cypermethrin-2	4.00	4.16	104	0.031	0.175	0.55
Cypermethrin-3	4.00	4.42	111	0.026	0.160	0.50
Cypermethrin-4	4.00	3.12	78.1	0.428	0.654	2.06
Deltamethrin	2.00	2.12	106	0.261	0.511	1.61
Esfenvalerate/Fenvalerate-1	2.00	2.10	105	0.044	0.210	0.66
Esfenvalerate/Fenvalerate-2	2.00	1.94	97.0	0.116	0.340	1.07
Lambda-cyhalothrin-1	2.00	1.71	85.5	0.066	0.256	0.81
Lambda-cyhalothrin-2	2.00	2.15	107	0.066	0.257	0.81
Permethrin-cis	6.00	5.89	98.1	0.125	0.353	1.11
Permethrin-trans	6.00	6.35	106	0.113	0.337	1.06
DBOB (Surrogate)	10.0	9.74	97.4	1.05	1.03	3.23

Students' t value for 99% confidence, where n=7, is 3.143

Determination of Pyrethroid Pesticides in Tissue and Sediment

1.0 Scope and Application

- 1.1 This method describes the sample preparation using an automated extraction system for the determination of trace residue levels of a selected list of pyrethroid pesticides in fish and shellfish tissues and sediments by dual column high resolution gas chromatography using electron capture detection. Table 1 lists the target pesticide compounds currently analyzed with their method detection limits and reporting limits. Detection limits were calculated using USEPA procedures found in Title 40 Code of Federal Regulations Part 136 (40CFR 136, Appendix B, revision 1.11). Sediment results can be found in Appendix A. The average percent recoveries, amount recovered and standard deviations are listed in Tables 4 and 5.
- 1.2 These procedures are applicable when low parts per billion analyses are required to monitor differences between burdens in organisms, soils and sediment from relatively uncontaminated reference areas and contaminated areas. In addition, the procedures are applicable when low detection limits are required for the estimation of potential health effects of bioaccumulated substances.

Table 1. Pyrethroid Compounds Analyzed, CAS Numbers, Minimum Detection Limits (MDL) and Reporting Limits (RL) in Sediment and Tissue.

Target Analytes	CAS#	Sediment		Tissue	
		MDL* ng/g Dry wt.	RL ng/g Dry wt.	MDL ng/g Wet wt.	RL g/g Wet wt.
Bifenthrin	82657-04-3	0.43	1.00	0.65	2.00
Cyfluthrin	68359-37-5	2.55	4.00	2.72	6.00
Cypermethrin	52315-07-8	2.44	4.00	1.66	4.00
Deltamethrin	52918-63-5	2.08	4.00	0.60	2.00
Esfenvalerate/ Fenvalerate	66230-04-4/ 51630-58-1	0.80	2.00	1.70	4.00
Fenpropathrin**	64257-84-7	2.00	4.00	1.35	4.00
Lambda- cyhalothrin	91465-08-6	1.15	4.00	1.49	4.00
Permethrin	52645-53-1	3.80	8.00	2.23	6.00

* Sediment MDL based on 50 percent moisture

-
- 1.3 This method can also be extended to include other pyrethroid pesticides. Table 2 lists these analytes, with their estimated method detection limits, estimated reporting limits and initial validation results in American River sediment (average percent recoveries and standard deviations for three replicates).
-

Table 2. Additional pyrethroid compounds, estimated MDL and RL, average percent recoveries and standard deviations in American River sediment; spike level 4 ng/g.

Pyrethroids by GC/ECD	Est. MDL (ng/g)	Est. RL (ng/g)	AVERAGE % Recovery	STD DEV
Allethrin	1.0	2.0	112	12.7
Flucythrinate	5.0	10	109	4.04
Phenothrin	5.0	10	92.6	13.0
Prallethrin	1.0	2.0	109	11.0
Tetramethin	5.0	10	118	10.7
Tralomethrin	5.0	10	93.0	1.45

2.0 Summary of Method

- 2.1 Sets of 12-16 homogenized tissue or sediment samples are scheduled for extraction by the project lead chemist. Extraction methods employed were developed and validated by the Water Pollution Control Laboratory (WPCL). Extract cleanup and partitioning methods are modifications of the multi-residue methods for fatty and non-fatty foods described in the U.S. Food and Drug Administration, Pesticide Analytical Manual, Vol. 1, 3rd Edition 1994, Chapter 3, Multi-residue Methods, Section 303-C1.

Homogenized tissue or sediment samples are removed from the freezer and allowed to thaw.

- 2.2 A 1-5 g (tissue or sediment homogenate) sample is weighed into a pre-weighed aluminum planchet and placed in a 70°C oven for 48 hours to determine moisture content. A 10 g sample is mixed using a clean glass stirring rod with approximately 7 g of pre-extracted Hydromatrix (Varian Corp) in a 250 mL Trace Clean Wide Mouth Jar until the mixture is free flowing. The mixture is then

poured into a 33 mL stainless steel Dionex Accelerated Solvent Extractor (ASE 200) extractor cell and packed by tamping the mixture. A solution containing DBCE surrogate is added to the cell and the cap is screwed onto the cell. The extractor cells (maximum of 24) are placed on the ASE 200 autosampler rack and the samples are extracted with a 50/50 mixture of acetone/dichloromethane (DCM) using heat and pressure. The extracts are automatically collected in 60 mL VOA vials.

- 2.3 The combined extracts are dried using sodium sulfate, evaporated to approximately 0.5 mL using Kuderna-Danish (K-D) glassware equipped with 3-ball Snyder columns and micro-Snyder apparatus and diluted to 10 mL using DCM. The extracts are then filtered through a 0.45 μ m syringe filter into J2 Scientific AccuPrep 170 (GPC) autosampler tubes.
- 2.4 The GPC autosampler tubes are then placed on the GPC autosampler for initial sample cleanup.
- 2.5 The cleaned-up extracts are evaporated using K-D apparatus and solvent exchanged into petroleum ether. The extracts are then fractionated using 25 grams of Florisil in a 11 mm x 300 mm column with a 250 mL reservoir. The Florisil columns are eluted with petroleum ether (PE) (Fraction 1), 6% diethyl ether/PE (Fraction 2) and 50% diethyl ether/PE (Fraction 3). The fractions are concentrated to an appropriate volume using K-D/micro K-D apparatus prior to analysis by dual column high resolution gas chromatography.

Table 3. Distribution of pyrethroid analytes among the Florisil fractions.

(0%) Fraction 1	(6%) Fraction 2	(50%) Fraction 3
DBOB (surrogate)	Bifenthrin	Cyfluthrin
(No pyrethroids elute in Fraction 1)	Permethrin	Cypermethrin
		Deltamethrin
		Fenpropathrin
		Es-fenvalerate
		Lambda-cyhalothrin
		DBCE (surrogate)

3.0 Interferences

- 3.1 Solvents, reagents, glassware, and other sample processing hardware may cause GC artifacts and/or elevated baselines, resulting in the misinterpretation of chromatograms. All materials should be demonstrated to be free from

interferences under the conditions of the analysis by running method blanks initially and with each sample lot. Specific selection of reagents and purification of solvents by distillation in all-glass systems are required. High-purity, distilled-in-glass solvents are commercially available.

An effective way of cleaning laboratory glassware is by rinsing with polar and non-polar solvents before use. The cleaning procedure used must be tested by analyzing procedural blanks prior to analyzing samples.

- 3.2 Phthalates are common laboratory contaminants that are used widely as plasticizers. Sources of phthalate contamination include plastic lab-ware, plastic tubing, plastic gloves, plastic coated glassware clamps, and have been found as a contaminant in Na_2SO_4 . Polytetrafluoroethylene (PTFE) can be used instead of polypropylene or polyethylene to minimize this potential source of contamination. However, use of PTFE lab-ware will not necessarily preclude all phthalate contamination.
- 3.3 Interferences co-extracted from tissue and sediment samples limit the method detection and quantitation limits. For this reason, sample extract cleanup is necessary to yield reproducible and reliable analyses of low level contaminants.

4.0 Apparatus and Materials

- 4.1 Wide mouth, borosilicate glass, pre-cleaned and certified, 250 mL, Qorpak or equivalent.
- 4.2 Chromatographic Column - (300 mm x 11 mm) borosilicate glass chromatography column with 250 mL reservoir and Teflon stopcock.
- 4.3 Glass wool, Pyrex - solvent washed prior to use.
- 4.4 Kuderna-Danish (K-D) Apparatus
 - 4.4.1 Concentrator tube - 10 mL, graduate (Kontes K0570050-1025, or equivalent). A ground stopper, 19/22 joint, is used to prevent evaporation of extracts.
 - 4.4.2 Evaporation flask - 500 mL (Kontes K-570050-0500, or equivalent), attached to concentrator tube with blue clamp (Kontes K-662750-0012).
 - 4.4.3 Snyder column - three ball (Kontes K-503000-0121, or equivalent).

- 4.4.4 Micro-Snyder column - (Kontes VWR KT569261-0319 or equivalent).
- 4.4.5 Boiling chips, Chemware[®] ultra-pure PTFE, extracted with acetone and petroleum ether. Note that boiling chips can be a significant source of contamination if not properly cleaned.
- 4.5 Water bath, Organomation Assoc. Inc. (OA-SYS/S-EVAP-KD), 115 V, thermostatically controlled with stainless steel cover to fit 5 K-D apparatus, installed in a fume hood.
- 4.6 Extractor, automated, Dionex Accelerated Solvent Extractor (ASE 200), Dionex P/N 047046.
 - 4.6.1 Extraction Cells, 33 mL, Dionex P/N 049562
 - 4.6.2 Filters, cellulose for ASE extraction cells, Dionex P/N 049458.
 - 4.6.3 VOA Vials, 60 mL, pre-cleaned and certified.
- 4.7 Sample vials - glass, 2 mL with PTFE-lined screw cap.
- 4.8 Analytical balance - capable of weighing 0.1 mg.
- 4.9 Drying oven.
- 4.10 Balance - capable of 100 g to the nearest 0.01 g.
- 4.11 Disposable Pasteur Pipets - (rinsed with solvents before use).
- 4.12 Aluminum dishes for moisture and lipid determination.
- 4.13 Desiccator with indicating desiccant.
- 4.14 Glass funnel, 75 mm.
- 4.15 Graduated cylinder, 250 mL and 100 mL.
- 4.16 Culture tubes, 16 x 100 mm, with PTFE lined cap.
- 4.17 Gas chromatographs (2), Hewlett-Packard HP 6890 plus, equipped with two micro ECD detectors with EPC, split-splitless injector with EPC, and autosampler.

- 4.18 Capillary columns, 60 meter DB5 and 60 meter DB17MS (J&W Scientific) (0.25 mm I.D. and 25 μ m film thickness) connected to a single injection port using a "Y" press fit connector.
- 4.19 Data System, Hewlett-Packard, to collect and record GC data, generate reports, and compute and record response factors for multi-level calibrations. Data system should be capable of calibrating a method using a minimum of 5 concentrations of analytical standards.
- 4.20 Homogenizer, Brinkman Polytron or equivalent equipped Teflon and titanium generator assembly (for homogenization of small sample amounts).
- 4.21 Gel Permeation (size exclusion) Chromatograph, automated, J2 Scientific AccuPrep 170, equipped with 70 g S-X3 BioBeads J2 Scientific P/N C0100 (100% DCM).

5.0 Reagents

- 5.1 Petroleum ether (PE), Burdick and Jackson, distilled in glass and pesticide residue or HRGC grade or equivalent.
- 5.2 Acetone. (Same as above).
- 5.3 Iso-Octane. (Same as above).
- 5.4 Diethyl ether preserved with 2% ethanol.(Same as above).
- 5.5 Dichloromethane (DCM). (Same as above).
- 5.6 Chem Elut-Hydromatrix, Varian P/N 0019-8003. Pre-extracted on ASE-200 with acetone/DCM prior to use.
- 5.7 Sodium sulfate. Anhydrous granular reagent grade, rinsed with PE prior to use.
- 5.8 Florisil, 60/100 mesh, PR grade, Floridin Corp.
- 5.9 Nitrogen, pre-purified grade (99.9999%) or better (used for ASE and GPC.)
- 5.10 Nitrogen, ultra-pure (99.99999%) for ECD makeup.
- 5.11 Helium, ultra-pure (99.99999%) for GC carrier gas.

- 5.12 Air, compressed, breathing quality, for ASE pneumatics.
- 5.13 Pyrethroid Surrogate Solution containing 40 ppb of DBCE obtained from AccuStd, New Haven, CT; P/N P-109S-H.
- 5.14 Pyrethroid Spiking Solution Mix: Individual compounds obtained from Chem Service, Inc., West Chester, PA. Solution made in acetone.

<u>Compound</u>	<u>Chem Service</u> <u>P/N</u>	<u>Concentration</u> <u>(ppb)</u>
Bifenthrin	PS2003	40
Cyfluthrin	F2460	80
Cypermethrin	PS1068	80
Deltamethrin	PS2071	80
Es-fenvalerate	PS2004	40
Fenpropathrin	PS2002	80
Lambda-cyhalothrin	PS2018	40
Permethrin	F2216S	100

- 5.15 Pyrethroid Instrument Calibration Standards: Individual compounds obtained from Chem Service (see 5.14) are mixed in iso-octane with concentrations ranging from 0.50 ppb to 50 ppb (based on bifenthrin).
- 5.16 Second Source Standards: Pyrethroid analytes were obtained from AccuStandard, New Haven, CT and Ultra Scientific, North Kingstown, RI for verification of calibration standards.

CAUTION

The toxicity or carcinogenicity of each compound or reagent used in this method has not been precisely determined. However, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of data handling Material Safety Data Sheets should also be made available to all personnel involved in these analyses.

6.0 Sample Collection, Preparation, and Storage

- 6.1 In the field, sources of contamination include sampling gear, grease from ship winches or cables, ship and/or motor vehicle engine exhaust, dust, and ice used for cooling. Efforts should be made to minimize handling and to avoid sources of contamination.
- 6.2 To avoid cross-contamination, all equipment used in sample handling should be thoroughly cleaned before each sample is processed. All instruments must be of a material that can be easily cleaned (e.g., stainless steel, anodized aluminum, or borosilicate glass). Before the next sample is processed, instruments should be washed with a detergent solution, rinsed with tap water, rinsed with a high-purity acetone, and finally rinsed with Type II water.

7.0 Sample Extraction

- 7.1 Remove sediment samples from freezer and allow to thaw. Prior to extraction, sediment samples are thoroughly mixed by hand using a clean glass rod or may be homogenized using a Polytron homogenizer equipped with stainless steel generator equipped with Teflon bearings. Sample sets of 12-16 should be extracted when possible. The ASE-200 extractor will extract 24 cells. Be sure to reserve enough cells for method blanks, matrix spikes, and laboratory control spikes.
- 7.2 A separate extraction bench sheet is started for each project, sample matrix type, and analysis type. Several bench sheets may be used for an extraction set.
- 7.3 Prepare a glass rod or Teflon spatula for each sample to be weighed by rinsing 3 times with petroleum ether using a Teflon wash bottle.
- 7.4 Label 60 mL VOA vials for the collection of the sample extract. The labels must be placed between 1.5" and 3" from the top of the VOA cap; if they are placed outside of this area, they will interfere with the ASE optical sensor. Use two VOA vials for each sample. Label the first VOA vial with the ASE position number, bench sheet number and the sample name. Label the second VOA vial the same but add "RE" to distinguish between the two vials. Label and weigh aluminum planchets for moisture determinations (samples ID can be made on the bottom of planchets using a ball point pen).
- 7.5 Tare a 250 mL glass jar. Using a clean (solvent rinsed) glass rod, stir the sediment so that the mixture is homogeneous. Weigh 10 g of sample into the

jar, record the weight on the bench sheet, and add the twice-extracted Hydromatrix from one ASE cell. Stir the mixture thoroughly and go on to the next sample. After approximately 15 minutes stir the sample again. Repeat this at 15 minute intervals two more times or until the sample mixture is free flowing.

- 7.6 Weigh 1-5 g of additional sample into a pre-weighed and tared aluminum planchet for % moisture analysis. Place planchets in 70°C oven for 48 hours and re-weigh dry weight.
- 7.7 Place a pre-rinsed powder funnel on top of a 33 mL ASE cell containing a pre-extracted cellulose filter (*the filter is the one that was used to pre-extract the Hydromatrix*).
- 7.8 Pour the sediment/Hydromatrix mixture through the powder funnel back into the extraction cell that the Hydromatrix was poured from. Tap the cell against the counter top to settle the contents. The mixture will fill the cell and it may be necessary to pack it slightly using the glass rod and the end of the powder funnel. The cells used for the **method blank** and **laboratory control spike** and its duplicate (*if used*) will contain only Hydromatrix.
- 7.9 **All** of the extraction cells are spiked with the pesticide surrogate standard. Spike each cell with exactly **1.0 mL** of the pesticide surrogate solution (40 ng/mL). Surrogate spikes must be witnessed, recorded and dated on the extraction bench sheet.
- 7.10 The extraction cells used for the matrix spike (MS) and duplicate matrix spike (MSD) and laboratory control spike (LCD) and its duplicate (LCSD) (*if used*) are spiked with exactly **1.0 mL** of the pesticide matrix spike solution (40 ng/mL). Matrix spikes must be witnessed, recorded and dated on the extraction bench sheet.
- 7.11 The extraction cells are capped (*Firmly tightened but do not overtighten*) and placed on the ASE 200 carousel. The first set of labeled VOA collection vials are placed on the ASE 200 collection carousel with the position numbers corresponding to the position numbers of the extraction cells. Make sure that the solvent reservoir contains enough solvent for the extraction.
- 7.12 Samples are extracted with acetone/methylene chloride (DCM) 50:50 using the following conditions:

Pre-heat	0 min.
Heat	5 min.
Static	5 min.

Flush	60%
Purge	300 sec.
Cycles	1
Pressure	1500 psi
Temp	100 °C
Sol A Other	100%

- 7.13 After the initial extraction is complete, remove full VOA vials and place in a Wheaton rack. Place the second set of collection VOA vials labeled "RE" on the ASE carousel. Check each of the extraction cells to make sure that the caps are (*firmly tightened*) as they tend to loosen with the first extraction. Make sure that the replacement vials are in the correct order. Make sure that the solvent reservoir contains enough solvent for the re-extraction. Re-start the ASE-200.
- 7.14 When extraction is completed, place VOA vials in a Wheaton rack with the "RE" vials next to the vials from the first extraction. The extracts should be re-capped with solid green caps (Qorpak) and placed in a refrigerator for storage until they are removed for the GPC cleanup procedure.

8.0 Gel Permeation Chromatography

IMPORTANT: *All glassware, glass wool, and sodium sulfate must be triple-rinsed with petroleum ether before they are used for this procedure.*

- 8.1 Remove VOA vials containing the sample extracts from the refrigerator. Make sure the vials are capped with the green Qorpak caps. Allow them to sit out until they are at room temperature.
- 8.2 Set up and label pre-cleaned K-D flasks (4-6) with concentrator tubes attached on ring stands in the fume hood. Place a funnel containing a plug of pre-cleaned glass wool in the bottom of the funnel and place the funnel in the top of the K-D flask. Add about two inches of pre-rinsed sodium sulfate to the funnel. Make sure that the level of the sodium sulfate is uniform across the funnel to prevent any possible splashing out.
- 8.3 Pour sample extracts from the VOA vials through sodium sulfate into the K-D flask. Add about 10 mL of DCM to the VOA vial, cap and shake and add this rinse to the sodium sulfate. Repeat with another 10 mL DCM rinse. Rinse the sodium sulfate with an additional portion of DCM (~50 mL) by pouring from a clean and rinsed 400 mL beaker. After the solvent has completely drained through the sodium sulfate add one more additional rinse of DCM (~50 mL)

from the beaker of clean DCM. Allow the DCM to completely drain through the sodium sulfate (~3-5 minutes).

- 8.4 Add 0.5 mL Iso-Octane using a macro-pipetter and a solvent rinsed boiling chip to each K-D flask. Place a Snyder column on the K-D flask and place the flask on the hot water bath set at 80-82°C. Evaporate the solvent until the reflux line falls below the top of the Snyder column. At this point there should be between 1-5 mL visible in the concentrator tube while the K-D apparatus is still on the hot water bath and 10 mL or less of the solvent remaining after the K-D flask is removed from the hot water bath and the solvent drains from the Snyder column. Dry off the water using a WyPall X60 towel to remove any water from around the ground glass union of the concentrator tube and the K-D flask to prevent any of it from entering the concentrator tube upon removal.
- 8.5 After the K-D apparatus has cooled and all of the solvent has drained from the Snyder column, remove the Snyder column, label the concentrator tube and then remove the concentrator tube from the flask and place the tube in a test tube rack and cover with pre-rinsed aluminum foil. Rinse the Snyder column with petroleum ether and place back in the column rack for storage. After all of the flasks have been removed from the hot water bath, repeat steps 2-5 for the remaining samples extracted with this set.
- 8.6 Add a new micro-boiling stone and place a clean micro-Snyder column on the concentrator tube with a blue clamp and place in a 400 mL beaker containing hot water heated to approximately 75°C on a hot plate. If the solvent does not begin to boil, remove the tube from the bath immediately, allow it to cool slightly, add a new micro boiling stone to prevent it from bumping and place it back in the bath. Evaporate the solvent until only 0.5 mL remains in the concentrator tube. Four or five tubes can be evaporated at one time.
- 8.7 When the solvent has been evaporated to 0.5 mL remove the tube from the bath and allow it to cool in a test tube rack. Remove the micro-Snyder column and add DCM to the concentrator tube to reach a final volume of 10.0 mL.
- 8.8 Draw the sample up into a clean 10 mL syringe with a 0.45 µm filter attached. Filter the sample into a 12 mL culture tube. Using a volumetric pipet remove 2.0 mL of the filtered sample and place it in a pre-weighed aluminum planchet for lipid determination. Cap the culture tube with the Teflon-insert style caps. Mark the bottom of the meniscus with a pen in case of evaporation before clean-up on GPC.
- 8.9 All samples are cleaned using a J₂ Scientific GPC (Autoinject 110, AccuPrep 170, DFW-20 Fixed Wavelength Detector, 1" ID glass column with 70g Bio-Beads SX-3 in 100% DCM)

8.9.1 From the desktop double click on the AccuPrep.exe shortcut to open the program. Click on the Use Injector button and allow the instrument time to initialize. Activate the pump by using the top left hand button. A solvent Control Pump window will open up. Click on the Apply Defaults button and then OK on the Selected Pressure Limit 30 psi. The pump should audibly be heard coming on and the green light should show that the system is on line and status flowing. Make sure that the bottle of clean DCM is full and the waste bottle is empty. Allow the system to pump for about 5 minutes before switching the column in-line (gray button next to Column that has 'Put in line' on it). The pressure will be observed to normally go up to the 12-16 psi range. Turn the power on to the detector to allow it at least 30 minutes of time to warm up before use. Because the scale is auto-adjusted in the software now it is no longer necessary to manually adjust the range on the unit itself.

8.9.2 While the system is equilibrating, the sequence can be entered. Click on the Seq button next to the Pump button. An 'Editing new sequence' window will pop up. This gives a view of the instrument which clearly shows the sample tray locations and the corresponding sample collection locations. By clicking on the sample tray position, a new window 'Adding sample at tray position #' will pop up. This allows information to be included about each specific sample. Sample position 1 will always be a calibration standard (CLP-340) which is run prior to any sequence of runs to verify instrument integrity. In the Sample ID field just type in 'CLP-340'. In the Descrip (optional), information pertaining to the project, laboratory control number, bench sheet number and date are typically added. The Method File needs to be changed to 'GPC-Sed' for only this sample and in the Sample Type field the 'Calibration' type can be chosen. After this information is completed click on the OK to continue. This returns you back to the main sequence window but now the first position will be highlighted in green. Continue by adding the next sample information to tray position 2, again following the same steps as before. The Method File name to be used is 'Sed-Pest'. Also by default, the Sample Type field will already be set at 'Sample'. This will not need to be changed until a duplicate sample (Duplicate), matrix spike (Matrix Spike), matrix spike duplicate (Spike Duplicate) and laboratory control spike (Spiked Blank) are encountered. After all the samples have been added to the sequence, save it as the WPCL log-in number (L-####-###). From the Editing sequence window print out the sample list. Compare the information to your original bench sheet to insure there are no mistakes. Make sure the GPC-Sed method is being used for the calibration standard and 'Sed-Pest' method is being used for the samples. Next verify that the samples are still at the marked line on the culture tubes (add DCM to the marked line if they are not). Place a tube with the GPC Calibration Standard Solution (CLP-340) in sample tray position 1 and then follow as the sequence was made in the remaining positions.

8.9.3 Get two boxes of the 125 mL Trace Clean amber bottles for sample collection. A bottle does not need to be placed in collection position #1 because that is the GPC Calibration Std (all goes to waste). Remove the white caps from the bottles and place them on top of the detector (so that Teflon side is not exposed to possible contamination). Label the boxes with laboratory control numbers and keep them for the post-GPC samples to be stored in. Now that the pump has had plenty of time to equilibrate the system and the detector has had plenty of time to warm up, in the Signal field click to adjust the setting to 'Absorbance Units' and click on the 'Zero Signal' button to set the baseline.

8.9.4 If the pressure seems to be pretty stable between the 12-16 psi range and all the samples positions and collection positions have been loaded, then click on the large button with the stop watch to begin the program. A window will pop up asking if the correct column method is loaded (100%DCM). Click on 'yes' to engage the syringe pump to begin priming. The sample probe will move over to sample position #1 and aspirate the sample. After the samples have all been processed (~1 hour per sample), remove the label from the sample position and place it on the bottle in corresponding collection position. Cap the bottle and place it back in the box that was retained for their storage. At the end of the sequence there will be a window that pops up saying that the 'Sequence has been successfully completed'. The column will switch offline and the pump will automatically shut down. The only thing that has to manually be turned off is the power to the detector.

8.10 Pour the GPC eluate into a rinsed K-D flask. Rinse the bottle with some DCM and add that to the K-D flask. Add 0.5 mL Iso-Octane and a micro boiling chip to each K-D flask. Attach a Snyder column to the flask and place in the hot water bath. When the volume of the solvent in the concentrator tube is level with the base of the K-D flask, lift the K-D apparatus up enough to be able to angle it slightly and add 40-50 mL Petroleum Ether through the top of the Snyder column. By holding the K-D apparatus at an angle, it allows the solvent to more easily drain back into the flask. Return to the K-D apparatus back into the hot water bath. Repeat this step 2 more times to successfully solvent exchange the sample from DCM to Petroleum Ether. When the apparent volume in the concentrator tube is 5-10 mL remove it from the hot water bath. Wipe down the K-D apparatus with a WyPall X60 towel especially around the ground glass junction. Remove the Snyder column from the K-D apparatus and allow to completely drain into the concentrator tube. After it has finished cooling, remove the concentrator tube and place a micro-Snyder column on it. Add a new micro boiling chip and place it in a 400 mL beaker containing water heated to approximately 75°C on a hot plate (4-5 tubes can be evaporated at one time). Evaporate the solvent down to 1-2 mL. Remove

it from the water bath and allow it to cool.

- 8.11 Transfer the solution to a 13 x 100 culture tube with a Pasteur pipette, rinse the concentrator tube with 0.5 ml of Petroleum Ether, vortex, and transfer the rinse to the culture tube. Repeat the rinse step two more times, and add each rinse to the culture tube. Cap the culture tube with a Teflon faced cap. Place extracts in a refrigerator for storage until the final Florisil column cleanup is done.
- 8.12 SEDIMENT SAMPLES ONLY: Check the GPC chromatogram for a sulfur peak. If a sulfur peak is present, add acid rinsed copper to the culture tubes to remove any residual sulfur from the extract.

9.0 Florisil Column Fractionation

IMPORTANT: *All glassware, glass wool, and sodium sulfate must be triple-rinsed with petroleum ether (PE) before they are used for this procedure. Florisil⁷ must be activated in an oven at 130°C for at least 24 hours prior to use.*

- 9.1 This procedure is performed after the GPC cleanup procedure for all sediment samples analyzed for pyrethroids.
- 9.2 Prepare the reagents to be used for Florisil cleanup: 6% ethyl ether in petroleum ether and 50% ethyl ether in PE. Make an amount slightly in excess of what is actually needed to allow for any loss which may occur during solvent transfer. The required volume is 200 mL per sample for the 6% (F2) and 220 ml per sample for the 50% (F3).
- 9.3 Prepare the chromatography columns. Place a small piece of PE rinsed glass wool in the bottom of the column and tap into place with a PE rinsed glass rod. Cover with a small portion (0.5 inch) of sodium sulfate. Fill the column with 5 grams of Florisil that has been measured using a dedicated pre-calibrated culture tube. Tap column with rubber "mallet" to firmly settle the Florisil. Top the column with 3/4-1 inch of sodium sulfate. This will prevent the column from being disrupted when solvent is added and will remove any residual water.
- 9.4 Place a 600 mL beaker under the column and pre-wet the column with about 25 mL of petroleum ether.

IMPORTANT: *From this point and through the elution process, the solvent level should never be allowed to go below the top of the sodium sulfate layer.*

- 9.5 When approximately 1 inch of PE remains above the surface of the column, place a K-D flask under column making sure that the stopcock is in the full open position. This will allow for a flow rate of about 2 to 3 mL/min. When the meniscus of the PE rinse reaches the column bed surface, introduce the sample on to the column using a long stem Pasteur pipette. Immediately add approximately 0.5 mL of PE to the tube, vortex, and add the rinse to the sample extract on the column. Add another 0.5 ml of PE to the tube, vortex, and add this final rinse to the sample extract on the column. Start the columns in a sequential fashion, and the lag time will be adequate to perform the necessary tasks for up to six columns.
- 9.6 When the combined sample and rinses reach the sodium sulfate layer, add 200 ml of Petroleum Ether that has been carefully measured out using a graduated cylinder to the column reservoir. Make sure that the stopcock is fully open in order to achieve the desired flow rate of 5 ml per minute. Collect directly into a clean PE rinsed 500 mL KD flask. This fraction contains surrogate DBOB only. No pyrethroid compounds elute in this fraction. See Table 3.
- 9.7 Just as the last of the F1 solvent reaches the top of the sodium sulfate layer, change the K-D flask, add 200 ml of the 6% diethyl ether/PE mixture to the column reservoir, replace with a second clean PE rinsed 500 mL KD flask, and elute as before. Add a micro boiling stone and attach a Snyder column with a green clamp to the K-D flask containing the 0% (F1) fraction and place vessel in the hot water bath with the temperature set at 80-82 °C and reduce volume to an apparent volume of 1 mL. Tap the Snyder column to make sure solvent is not trapped between the balls then remove the vessel from the bath and place in the vessel stand to cool.
- 9.8 Repeat the above using 220 mL of 50% diethyl ether/PE mixture.
- 9.9 When the vessels are cool, remove the concentrator tube from the K-D flask add a new micro boiling stone and attach a clean micro-Snyder column to the concentrator tube with a blue clamp and place in a 400 mL beaker containing hot water heated to approximately 75°C on a hot plate. Evaporate the solvent until only 0.5-1 mL remains in the concentrator tube. Four or five tubes can be evaporated at one time.
- 9.10 When the solvent has been evaporated to 0.5-1 mL remove the tube from the bath and allow it to cool in a test tube rack. Remove the micro-Snyder column and add iso-octane to the concentrator tube to reach a final volume of 2.0 mL. Mix the tube contents by tapping the bottom of the tube causing a vortex which will rinse the sides of the tube. A Vortex Genie mixer may be used for this step. Transfer the extract to a clean labeled culture tube and cap.

9.11 Repeat for 6% (F2) and 50% (F3) extracts. The extracts are ready for analysis by GC-ECD.

10.0 Analytical Procedure

10.1 Before the sample extracts can be analyzed by the GC-ECD, a sequence listing the order of calibration standards, second source check standards, initial and continuing calibration blanks, initial and continuing calibration verification standards and sample extracts is written using Agilent Chemstation Software or Varian (GC/MS/MS) software.

10.2 Each GC sequence includes a minimum of seven calibration standards. The calibration curve concentration for pyrethroids differs for different analytes, but in general the range is 1 ppb to 100 ppb.

10.3 To verify the calibration standards, second source pesticide check standards (see 5.16) are analyzed.

10.4 An initial calibration blank and initial calibration verification standard is analyzed after the calibration standards and prior to the first sample extract. Continuing calibration blanks (CCBs) and calibration verification standards (CCVs) are analyzed after ten sample extracts have been analyzed. If a CCV fails, the five samples prior to the failed CCV and the five samples after the failed CCV are re-analyzed after a new calibration curve is analyzed.

10.5 The CCV analyte concentrations are mid-range of the calibration curve (20-60 ppb).

10.6 As the run proceeds, sample extracts are monitored for analyte concentrations that are greater than the calibration curve and need dilution.

10.7 Instrumentation

10.7.1 Agilent 6890*plus* gas chromatograph equipped with two ^{63}Ni micro-electron capture detectors with EPC and autosampler. Two 60 meter, 0.25 mm ID, 0.25 μm (film thickness) fused silica columns (J&W) are used. A 5 meter length of DB-5 column is connected to a press fit "Y" union which splits the column effluent into two 60 m columns, a DB-5 and a DB-17MS. The injector is a split-splitless injector with EPC.

10.7.2 Chromatograph conditions: The injector is operated isothermal at 240°C. The oven has an initial temperature of 130°C which is held for 1 minute and then temperature programmed to 240°C at a rate of

20°C/min and held for 7 min. It is then programmed to 290°C at a rate of 2°C/min and is held for 16.50 min. Helium is used as the carrier gas at a linear velocity of 33 cm/sec. Nitrogen is used for the detector makeup at 30 mL/min. See Table 3 for elution order of pyrethroid analytes.

- 10.7.3 Sample volume: Three microliters of samples and standards are injected and split approximately 50/50 onto the 60 m DB-5 and the 60 m DB-17.MS
- 10.7.4 Data processing: An Agilent 3365 Series II Chemstation is used for detector signal acquisition and analysis.

Table 3. Elution times of pyrethroid analytes from J&W 60 meter columns on GC/ECD.

Pyrethroids	Retention Time (minutes)	
	DB-5	DB-17MS
Bifenthrin	21.47	23.57
Cyfluthrin-1	30.26	36.15
Cyfluthrin-2	30.63	36.51
Cyfluthrin-3	30.98	36.90
Cyfluthrin-4	31.13	NA
Cypermethrin-1	31.58	38.28
Cypermethrin-2	31.98	38.70
Cypermethrin-3	32.32	39.08
Cypermethrin-4	32.47	NA
Deltamethrin	38.72	49.50
Es-fenvalerate-1	35.54	43.79
Es-fenvalerate-2	36.42	45.12
Fenpropathrin	20.85	26.10
Lambda-cyhalothrin-1	24.25	27.29
Lambda-cyhalothrin-2	24.98	28.11
Permethrin-1	27.88	34.12
Permethrin-2	28.38	34.73
DBOB (surrogate)	8.60	8.13
DBCE (surrogate)	22.82	25.88

11.0 Method Performance

- 11.1 Pyrethroid analyte average percent recoveries, amount recovered and standard deviations in American River sediment and American River Hatchery trout are listed in Tables 4 and 5, respectively.

Table 4. Method validation results for pyrethroids in American River sediment; spike level at 5-20 ng/g.

Pyrethroids by GC/ECD	Average % Recovery	Average Amount Recovered	Standard Deviation (n=8)
Bifenthrin	106	5.30	0.14
Cyfluthrin	108	10.4	0.85
Cypermethrin	108	11.1	0.81
Deltamethrin	62.0	3.10	0.69
Es/Fenvalerate	107	5.39	0.27
Lambda- cyhalothrin	104	5.17	0.38
Permethrin	99.0	16.2	1.27

Table 5. Method validation results for pyrethroids in American River Hatchery trout; spike level at 4-16 ng/g.

Pyrethroids by GC/ECD	Average % Recovery	Average Amount Recovered	Standard Deviation (n=8)
Bifenthrin	88.1	3.52	0.207
Cyfluthrin	98.7	15.8	0.865
Cypermethrin	74.3	5.94	0.528
Deltamethrin	41.8	1.67	0.191
Es/Fenvalerate	52.8	2.11	0.534
Fenprothrin	85.0	3.40	0.428
Lambda-cyhalothrin	89.1	3.56	0.473
Permethrin	97.2	11.7	0.708

12.0 References

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SOP Section Approval: _____

Date: _____

SOP Final Approval: _____

Date: _____

SOP QA Officer Approval: _____

Date: _____

APPENDIX A**METHOD DETECTION LIMIT VALIDATION FOR PYRETHROIDS IN SEDIMENT**

Pyrethroid Pesticides	Spike Amount Expected (ng/g)	Average Amount Recovered (ng/g)	Average Percent Recovery	Variance Amount Recovered (ng/g)	Standard Deviation Amount Recovered	MDL=t*Stdev (ng/g)
Bifenthrin	5.00	5.30	106	0.021	0.143	0.43
Cyfluthrin-1	10.0	10.4	104	0.723	0.850	2.55
Cyfluthrin-2	10.0	10.8	108	1.16	1.08	3.23
Cyfluthrin-3	10.0	10.6	106	0.421	0.649	1.94
Cyfluthrin-4	10.0	11.2	112	1.47	1.21	3.64
Cypermethrin-1	10.0	11.1	111	0.662	0.814	2.44
Cypermethrin-2	10.0	10.9	109	0.916	0.957	2.87
Cypermethrin-3	10.0	10.4	104	0.665	0.815	2.44
Cypermethrin-4	10.0	11.0	110	0.467	0.683	2.05
Deltamethrin	5.00	3.10	62	0.481	0.693	2.08
Esfenvalerate/Fenvalerate-1	5.00	5.39	108	0.072	0.268	0.80
Esfenvalerate/Fenvalerate-2	5.00	5.31	106	0.126	0.355	1.06
Lambda-cyhalothrin-1	5.00	5.17	103	0.147	0.384	1.15
Lambda-cyhalothrin-2	5.00	5.26	105	0.070	0.265	0.79
Permethrin-cis	15.0	16.2	108	1.60	1.27	3.80
Permethrin-trans	15.0	13.5	90	9.28	3.05	9.13

American River sediment was used for validation (29.2 % moisture)

Students' t value for 99% confidence, where n=8, is 2.998

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Title: Analysis of Pyrethroids in Sediment Water

1. Scope:

This section method (SM) documents selective pyrethroids analysis in sediment water and is followed by all authorized Environmental Analysis 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 water. The pyrethroids are extracted from the sediment water using liquid-liquid extraction with hexane. The extracts are concentrated and then cleaned up 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 resmethrin. 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 15 ppt reporting limits.

3. Safety:

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

3.2 Hexane is a flammable and toxic solvent; it should be handled with care in a ventilated area.

4. Interferences:

4.1 Glassware cleaning is extremely critical. It was determined that glassware needed to be rinsed with hexane prior to use. The glassware cleaning procedure was tested by analyzing solvent blanks prior to sample extraction.

4.2 The electron capture detector (ECD) is not truly an element specific detector, it will also respond to compounds containing S, NO₂ or conjugated C=O functional groups.

5. Apparatus and Equipment:

5.1 Rotary Evaporator (Buchi/Brinkman or equivalent)

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- 5.2 Nitrogen evaporator (Meyer N-EVAP Organomation Model #112 or equivalent)
- 5.3 Balance, (Mettler PC 4400 or equivalent)
- 5.4 Vortex-vibrating mixer
- 5.5 Gas Chromatograph (GC) equipped with ⁶³Ni ECD detectors
- 5.6 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 Hexanes, nanograde or equivalent pesticide grade
- 6.13 Diethylether, nanograde or equivalent pesticide grade
- 6.14 Separatory funnel, 2 L
- 6.15 Boiling flask, 500 mL
- 6.16 Sodium Sulfate, ACS grade
- 6.17 Funnels, short stem, 60°, 10 mm diameter
- 6.18 Glass wool, Pyrex® fiberglass slivers 8 microns
- 6.19 Beaker, 1 L
- 6.20 Florisil SPE cartridge, 2 grams with 20 mL reservoir
- 6.21 Volumetric Pipette, 1 mL
- 6.22 Test tube, 50 mL
- 6.23 Test tube, 15 mL
- 6.24 Disposable Pasteur pipettes, and other laboratory ware as needed
- 6.25 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

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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/ μ L with hexanes for identification purposes.

A combination standard of 10 μ g/mL was prepared from individual mg/mL standards with acetone to be used for fortification. Another 10 μ g/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/ μ L 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 separate refrigerator (32-40 °F)

9. Test Sample Preparation:

9.1 Background Preparation

The Department of Pesticide Regulation (DPR) provided the sediment water for background to be used in method validation and QC. The sediment water was prepared by adding 5 g of soil to approximately a liter of American river water.

9.2 Spike

Take a liter of background from refrigerator and allow it to come to room temperature. Fortify at a level requested by client. After fortification mix well and process same as samples.

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9.3 Test Sample Extraction

- 9.3.1 Remove water samples from refrigerator and allow samples to come to room temperature before weighing them. Record weight.
- 9.3.2 Transfer the water sample to a 2 L separatory funnel leaving as much of the sediment as possible in the sample bottle.
- 9.3.3 Add 60 mL of hexanes to the sample bottle and manually shake for 30 seconds.
- 9.3.4 Transfer hexane and sediment into the separatory funnel and shake for 2 min, venting frequently.
- 9.3.5 Allow the layers to separate, drain the lower aqueous layer into a 1L beaker. Pour the hexane layer through a funnel containing a plug of glass wool and approximately 40 g sodium sulfate into a 500 mL boiling flask. (sodium sulfate had been pre-rinsed with hexane before use)
- 9.3.6 Transfer the water from the beaker into the separatory funnel and repeat steps 9.3.3 – 9.3.6 two more times shaking for 1 min. Combine the extracts in the same boiling flask. Record sample bottle weight.
- 9.3.7 Rotary evaporate to ~ 5 mL under vacuum at approximately 17-22 inch Hg in a water bath at 44-47° C.
- 9.3.8 Transfer the extract to a 15 mL test tube. Rinse flask 3 times with approximately 2 mL of hexane and transfer each rinsate to the same test tube.
- 9.3.9 Place the test tube on a nitrogen evaporator under a gentle stream of nitrogen with water bath set at 40-45° C and concentrate to ~ 2 mL final volume.

Cleanup

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

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9.3.11 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.

9.3.12 Elute the pesticides from the cartridge with 30 mL of 15% diethylether in hexane and collect in a 50 mL tube.

9.3.13 Evaporate the sample eluants to dryness under a gentle stream of nitrogen in a 40-45° C water bath.

9.3.14 Pipet 1mL of hexane into the test tube and vortex well. Vial extract into 3 autosampler vials with inserts.

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 100 °C, hold 0 min., ramp at 10 °C/min to 230 °C and hold for 5 min, ramp at 2° C/min to final temperature of 280° for 7 min.; injection volume 2 μL. Flow rates of the Helium carrier gas

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were 3.8 mL/min. and 1.8 mL/min. for the HP-5MS and the DB-608 columns, respectively.

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 time 11.55 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 water samples are spiked at 10 ppt except resmethrin, which was spiked at 20 ppt 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:

$$\text{MDL} = tS$$

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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 bifenthrin is 5 ppt and for all other compounds it is 15 ppt. This reporting limit is conservatively set at 15 ppt for most of the compounds after taking into account the matrix effect and various sample backgrounds that could be encountered.

12.3 Method Validation

The method validation consisted of five sample sets. Each set included four levels of fortification (20, 50, 250 and 1000 ppt,) 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 ± 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 ± 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.

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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.

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

14. Reporting Procedure:

14.1 The ECD HP-5ms is used as the primary column for reporting results for the pyrethroids. Resmethrin results are reported from the MSD since it doesn't chromatograph 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.

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 15 ppt level for

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fenprothrin, cyfluthrin, cypermethrin, fenvalerate/esfenvalerate and deltamethrin.

- 15.3 A storage stability study was done with this project. The storage stability study consisted of a 100 ppt spike concentrate and 3 replicates over 28 day period. Fifteen bottles containing background water with sediment added were spiked and stored in the refrigerator until analyzed on 0, 4, 7, 14, and 28 days. Along with the storage spikes a blank and method control spike were extracted. This same procedure was repeated again, but this time 900 mL of sediment water was spiked and 10 mL of hexane was added to each bottle as a keeper. The amount spiked was kept the same making the level 111 ppt. The study showed rapid degradation for resmethrin, deltamethrin, and permethrin trans by day 4. The other compounds show gradual degradation. The bottles where the keeper was added showed little or no degradation for all the compounds up to day 28. A previous storage study was done analyzing bifenthrin, lambda cyhalothrin, permethrin, cyfluthrin, cypermethrin and fenvalerate/esfenvalerate. The results from that study showed degradation for permethrin and lambda cyhalothrin by day 3. No keeper was added in that study. Results from the current storage study are presented in Appendix 3.
- 15.4 The sample matrix may require that the liner be changed more frequently and the column trimmed to maintain sensitivity.
- 15.5 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 Organochlorine 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)

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16.3 J. White, H. Feng, Determination of Pyrethroids in Sediment Water,
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Appendix 1

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

Spike level is 10 ppt for all compounds except Resmethrin, which is 20 ppt

	Bifenthrin	Fenopropathrin	Lambda cyhalothrin epimer	Lambda cyhalothrin	Permethrin cis	Permethrin trans
	ppt	ppt	ppt	ppt	ppt	ppt
blk sed	n/d	n/d	n/d	n/d	n/d	n/d
spk1	8.78	9.94	9.50	9.73	9.44	8.16
spk2	9.39	10.9	10.3	10.5	10.5	8.83
spk 3	8.61	10.6	9.61	9.79	12.1	12.2
spk 4	7.95	9.95	9.85	9.91	12.5	14.6
spk 5	9.54	11.2	10.2	10.6	10.8	12.4
spk 6	9.06	10.7	10.1	10.4	9.89	8.62
spk 7	9.39	10.9	10.4	10.4	10.4	9.61
Std dev	0.561	0.484	0.348	0.366	1.12	2.44
MDL	1.76	1.52	1.09	1.15	3.52	7.68
RL	5.00 ppt	15.0 ppt	15.0 ppt	15.0 ppt	15.0 ppt	15.0ppt

	Cyfluthrin	Cypermethrin	Fenvalerate/Esfenvalerate	Deltramethrin	Resmethrin
	ppt	ppt	ppt	ppt	ppt
blk sed	n/d	n/d	n/d	n/d	n/d
spk1	11.1	10.7	11.5	8.60	16.7
spk2	11.7	11.7	12.5	8.83	15.9
spk 3	11.1	10.5	11.6	7.92	16.1
spk 4	10.7	10.4	12.1	7.65	16.4
spk 5	11.6	11.0	12.8	9.04	16.7
spk 6	11.6	11.2	12.7	8.81	17.6
spk 7	12.4	11.8	12.8	9.26	13.7
Std dev	0.551	0.556	0.558	0.590	1.22
MDL	1.73	1.75	1.75	1.86	3.82
RL	15.0 ppt	15.0 ppt	15.0 ppt	15.0 ppt	15.0 ppt

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Appendix 2

Method Validation Data and Control Limits

	Bifenthrin	Fenpropathrin	λ Cyhalothrin epimer	λ Cyhalothrin	Permethrin cis	Permethrin trans
Spike Level (ppt)	Recovery(%)	Recovery(%)	Recovery(%)	Recovery(%)	Recovery(%)	Recovery(%)
20	80.5	93.0	92.0	90.5	86.5	90.0
	86.0	92.0	90.5	88.0	89.0	90.5
	86.5	104	100	103	103	104
	83.0	101	97.5	101	96.0	105
	78.0	92.5	91.5	94.0	88.5	91.0
50	82.8	96.2	97.2	95.4	94.4	91.2
	82.2	97.6	91.2	90.6	87.6	90.2
	97.2	113	112	112	113	118
	72.2	100	93.0	94.6	88.2	93.6
	93.4	108	106	109	106	111
250	87.2	99.2	96.4	94.4	93.2	94.0
	74.4	91.6	84.8	85.2	84.8	85.6
	94.0	110	106	105	108	115
	94.8	99.2	99.6	98.4	102	105
	79.6	94.8	89.6	89.9	90.0	91.2
1000	76.2	86.4	82.9	81.9	78.8	80.2
	78.9	98.9	88.8	90.3	88.4	90.1
	94.1	109	109	106	105	111
	96.3	105	102	102	104	105
	82.1	94.7	91.7	89.9	89.8	90.5
Mean	85.0	99.3	96.1	95.9	94.8	97.6
SD	7.71	7.07	7.95	8.20	9.25	10.9
UCL	108	121	120	121	123	130
UWL	100	113	112	112	113	119
LWL	69.6	85.2	80.2	79.5	76.3	75.8
LCL	61.8	78.1	72.2	70.9	67.1	65.7

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Appendix 2 (cont..)

	Cyfluthrin	Cypermethrin	Fenvalerate/ Esfenvalerate	Deltamethrin	Resmethrin
Spike Level (ppt)	Recovery(%)	Recovery(%)	Recovery(%)	Recovery(%)	Recovery(%)
20.0	92.0	92.0	92.0	89.0	76.0
	87.0	87.0	86.5	81.5	97.0
	112	117	116	111	85.0
	103	96.0	109	103	116
	92.0	90.5	98.5	103	89.5
50	97.2	97.2	88.6	96.8	89.6
	90.6	90.6	89.0	79.6	96.0
	125	127	110	118	98.2
	103	95.0	87.8	100	93.8
	115	117	116	123	97.6
250	90.8	90.8	89.6	86.0	98.8
	82.8	82.8	79.2	70.0	75.6
	115	122	122	120	93.2
	108	110	111	103	109
	93.2	90.8	93.6	88.4	104
1000	79.7	79.7	79.8	75.5	62.8
	88.7	88.7	87.1	84.8	82.6
	111	122	115	105	97.0
	107	110	107	101	95.1
	92.9	82.9	85.4	89.4	99.1
Mean	99.6	99.5	98.2	96.4	92.8
SD	12.3	14.9	13.6	14.9	12.1
UCL	136	144	139	141	129
UWL	124	129.3	125	126	117
LWL	75.0	69.7	71.0	66.6	68.6
LCL	63.6	54.7	57.3	51.6	56.6

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Appendix 3

Storage Stability Project

Matrix: American River Water with 5 g sediment added

Spike Level:100ppt

Keeper: 10 mL hexane

Bifenthrin

spk	day 0		day 4		day 7		day 14		day 28	
	ppt	%	ppt	%	ppt	%	ppt	%	ppt	%
1	92.4	92.4	76.2	76.2	67.3	67.3	63.1	63.1	51.5	51.5
2	77.4	77.4	80.4	80.4	69.0	69.0	65.0	65.0	54.7	54.7
3	90.3	90.3	76.2	76.2	62.4	62.4	54.9	54.9	58.3	58.3
Mean		86.7		77.6		66.2		61.0		54.8

Bifenthrin with Keeper

spk	day 0		day 4		day 7		day 14		day 28	
	ppt	%	ppt	%	ppt	%	ppt	%	ppt	%
1	105	94.60%	96.5	86.9%	93.4	84.1%	88.4	79.6%	87.2	78.6%
2	104	93.70%	95.6	86.1%	89.4	80.5%	75.0	67.6%	98.0	88.3%
3	108	97.30%	99.9	90.0%	80.4	72.4%	87.5	78.8%	92.6	83.4%
Mean		95.20%		87.7%		79.0%		75.3%		83.4%

Fenpropathrin

spk	day 0		day 4		day 7		day 14		day 28	
	ppt	%	ppt	%	ppt	%	ppt	%	ppt	%
1	99.1	99.1	92.6	92.6	84.1	84.1	73.0	73.0	74.9	74.9
2	94.5	94.5	99.4	99.4	85.7	85.7	88.4	88.4	78.9	78.9
3	103	103	97.9	97.9	88.4	88.4	65.0	65.0	79.0	79.0
Mean		98.9		96.6		86.1		75.5		77.6

Fenpropathrin with Keeper

spk	day 0		day 4		day 7		day 14		day 28	
	ppt	%	ppt	%	ppt	%	ppt	%	ppt	%
1	111	100%	109	98.2%	107	96.4%	103	92.8%	102	91.9%
2	109	98.2%	111	100%	109	98.2%	90.9	81.9%	106	95.5%
3	117	105%	113	102%	105	94.6%	107	96.4%	110	99.1%
Mean		101.1%		100.1%		96.4%		90.4%		95.5%

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Lambda Cyhalothrin Epimer

spk	day 0		day 4		day 7		day 14		day 28	
	ppt	%	ppt	%	ppt	%	ppt	%	ppt	%
1	100	100	86.8	86.8	73.7	73.7	70.8	70.8	52.9	52.9
2	93.3	93.3	90.1	90.1	75.5	75.5	71.7	71.7	56.1	56.1
3	101	101	85.1	85.1	71.8	71.8	61.6	61.6	53.5	53.5
Mean		98.1		87.3		73.7		68.0		54.2

Lambda Cyhalothrin Epimer with Keeper

spk	day 0		day 4		day 7		day 14		day 28	
	ppt	%	ppt	%	ppt	%	ppt	%	ppt	%
1	114	103%	104	93.7%	102	91.9%	97.0	87.4%	94.9	85.5%
2	111	100%	105	94.6%	99.6	89.7%	86.2	77.7%	105	94.6%
3	118	106%	108	97.3%	92.0	82.9%	94.7	85.3%	99.8	89.9%
Mean		103.0%		95.2%		88.2%		83.5%		90.0%

Lambda Cyhalothrin

spk	day 0		day 4		day 7		day 14		day 28	
	ppt	%	ppt	%	ppt	%	ppt	%	ppt	%
1	99.6	99.6	82.1	82.1	74.0	74.0	70.1	70.1	50.8	50.8
2	90.3	90.3	85.3	85.3	76.8	76.8	70.9	70.9	54.4	54.4
3	100	100	83.1	83.1	73.2	73.2	62.1	62.1	53.2	53.2
Mean		96.6		83.5		74.7		67.7		52.8

Lambda Cyhalothrin with Keeper

spk	day 0		day 4		day 7		day 14		day 28	
	ppt	%	ppt	%	ppt	%	ppt	%	ppt	%
1	111	100%	102	91.9%	100	90.1%	92.2	83.1%	91.8	82.7%
2	111	100%	100	90.1%	97.5	87.8%	84.2	75.9%	101	91.0%
3	115	104%	104	93.7%	93.1	83.9%	91.5	82.4%	96.2	86.7%
Mean		101.3%		91.9%		87.3%		80.5%		86.8%

Permethrin cis

spk	day 0		day 4		day 7		day 14		day 28	
	ppt	%	ppt	%	ppt	%	ppt	%	ppt	%
1	98.8	98.8	82.0	82.0	69.2	69.2	67	67	42.2	42.2
2	91.4	91.4	80.3	80.3	72.4	72.4	65.6	65.6	48.3	48.3
3	100	100	79.3	79.3	69.5	69.5	57.7	57.7	38.3	38.3
Mean		96.6		80.5		70.4		63.4		42.9

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Permethrin cis with Keeper

spk	day 0		day 4		day 7		day 14		day 28	
	ppt	%	ppt	%	ppt	%	ppt	%	ppt	%
1	110	99.1%	99.5	89.6%	105	94.6%	96.3	86.8%	95.7	86.2%
2	111	100%	102	91.9%	100	90.1%	88.4	79.6%	106	95.5%
3	113	102%	103	92.8%	93.8	84.5%	93.7	84.4%	102	91.9%
Mean		100.3%		91.4%		89.7%		83.6%		91.2%

Permethrin trans

spk	day 0		day 4		day 7		day 14		day 28	
	ppt	%	ppt	%	ppt	%	ppt	%	ppt	%
1	107	107	66.0	66.0	47.9	47.9	37.9	37.9	9.53	9.53
2	100	100	65.7	65.7	47.3	47.3	34.4	34.4	22.4	22.4
3	106	106	64.3	64.3	50.8	50.8	30.3	30.3	11.9	11.9
Mean		104.3		65.3		48.7		34.2		14.6

Permethrin trans with Keeper

spk	day 0		day 4		day 7		day 14		day 28	
	ppt	%	ppt	%	ppt	%	ppt	%	ppt	%
1	120	108%	101.1	91.0%	108	97.3%	96.9	87.3%	99.5	89.6%
2	118	106%	105	94.6%	102	91.9%	90.2	81.3%	110	99.1%
3	123	111%	106	95.5%	97.0	87.4%	98.7	88.9%	103	92.8%
Mean		108.3%		93.7%		92.2%		85.8%		93.8%

Cyfluthrin

spk	day 0		day 4		day 7		day 14		day 28	
	ppt	%	ppt	%	ppt	%	ppt	%	ppt	%
1	103	103	79.5	79.5	65.3	65.3	62.3	62.3	41.3	41.3
2	97.9	97.9	80.4	80.4	68.1	68.1	60.2	60.2	45.1	45.1
3	106	106	77.6	77.6	68.6	68.6	54.3	54.3	41.8	41.8
Mean		102.3		79.2		67.3		58.9		42.7

Cyfluthrin with Keeper

spk	day 0		day 4		day 7		day 14		day 28	
	ppt	%	ppt	%	ppt	%	ppt	%	ppt	%
1	113	102%	96.1	86.6%	105	94.6%	92.0	82.9%	91.6	82.5%
2	115	104%	96.3	86.8%	98.6	88.8%	84.7	76.3%	99.1	89.3%
3	121	109%	93.9	84.6%	93.6	84.3%	84.7	76.3%	97.8	88.1%
Mean		105.0%		86.0%		89.2%		78.5%		86.6%

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Cypermethrin

spk	day 0		day 4		day 7		day 14		day 28	
	ppt	%	ppt	%	ppt	%	ppt	%	ppt	%
1	103	103	80.5	80.5	66.9	66.9	65.2	65.2	44.7	44.7
2	96.8	96.8	74.0	74.0	64.7	64.7	62.7	62.7	48.5	48.5
3	104	104	77.3	77.3	62.6	62.6	55.3	55.3	41.3	41.3
Mean		101.3		77.3		64.7		61.1		44.8

Cypermethrin with Keeper

spk	day 0		day 4		day 7		day 14		day 28	
	ppt	%	ppt	%	ppt	%	ppt	%	ppt	%
1	115	104%	88.6	79.8%	105	94.6%	90.4	81.4%	83.9	75.6%
2	115	104%	91.0	82.0%	98.9	89.1%	83.5	75.2%	95.5	86.0%
3	121	109%	91.0	82.0%	93.6	84.3%	83.5	75.2%	94.8	85.4%
Mean		105.7%		81.3%		89.3%		77.3%		82.3%

Fenvalerate / Esf

spk	day 0		day 4		day 7		day 14		day 28	
	ppt	%	ppt	%	ppt	%	ppt	%	ppt	%
1	106	106	79.0	79.0	66.8	66.8	68.9	68.9	51.7	51.7
2	99.8	99.8	78.6	78.6	70.8	70.8	65.8	65.8	26.6	26.6
3	107	107	75.7	75.7	67.3	67.3	58.8	58.8	55.2	55.2
Mean		104.3		77.8		68.3		64.5		44.5

Fenvalerate / Esf with Keeper

spk	day 0		day 4		day 7		day 14		day 28	
	ppt	%	ppt	%	ppt	%	ppt	%	ppt	%
1	121	109%	93.5	84.2%	105	94.6%	91.0	82.0%	86.9	78.3%
2	120	108%	84.7	76.3%	97.9	88.2%	83.6	75.3%	93.1	83.9%
3	124	112%	86.7	78.1%	92.1	83.0%	83.6	75.3%	91.4	82.3%
Mean		109.7%		79.5%		88.6%		77.5%		81.5%

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Deltamethrin

spk	day 0		day 4		day 7		day 14		day 28	
	ppt	%	ppt	%	ppt	%	ppt	%	ppt	%
1	96.8	96.8	60.6	60.6	47.8	47.8	47.9	47.9	31.6	31.6
2	88.7	88.7	60.9	60.9	56.7	56.7	46.8	46.8	32.5	32.5
3	95.1	95.1	56.8	56.8	49.3	49.3	35.1	35.1	34.0	34.0
Mean		93.5		59.4		51.3		43.3		32.7

Deltamethrin with Keeper

spk	day 0		day 4		day 7		day 14		day 28	
	ppt	%	ppt	%	ppt	%	ppt	%	ppt	%
1	106	95.5%	78.1	70.4%	86.5	77.9%	75.0	67.6%	74.1	66.8%
2	110	99.1%	68.0	61.3%	83.8	75.5%	67.2	60.5%	78.6	70.8%
3	113	102%	72.5	65.3%	77.7	70.0%	75.9	68.4%	78.1	70.4%
Mean		98.9%		65.7%		74.5%		65.5%		69.3%

Resmethrin

spk	day 0		day 4		day 7		day 14		day 28	
	ppt	%	ppt	%	ppt	%	ppt	%	ppt	%
1	91.2	91.2	52.0	52.0	58.3	58.3	47.0	47.0	27.6	27.6
2	92.9	92.9	42.7	42.7	60.5	60.5	45.4	45.4	37.0	37.0
3	90.8	90.8	57.2	57.2	51.9	51.9	42.9	42.9	26.3	26.3
Mean		91.6		50.6		56.9		45.1		30.3

Resmethrin with Keeper

spk	day 0		day 4		day 7		day 14		day 28	
	ppt	%	ppt	%	ppt	%	ppt	%	ppt	%
1	117	105%	86.6	78.0%	104	93.7%	98.8	89.0%	98.8	89.0%
2	109	98.2%	85.0	76.6%	96.8	87.2%	95.9	86.4%	88.7	79.9%
3	115	104%	90.1	81.2%	98.9	89.1%	98.6	88.8%	98.5	88.7%
Mean		102.4%		78.6%		90.0%		88.1%		85.9%

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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 orbital 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, NO₂ 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 ⁶³Ni ECD detectors

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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:

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- 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

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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 ± 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.

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- 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 Rotary 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.

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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/ μ L.
- 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 μ L. 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.

11.3 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 μ L. Flow rate of Helium carrier gas was 1.0 mL/min.

Ions Selected for SIM Acquisition:

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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 time 11.55 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:

$$\text{MDL} = tS$$

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 pyrethroids 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 backgrounds that could be encountered.

12.3 Method Validation

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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 ± 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 ± 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.

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

14. Reporting Procedure:

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- 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.
 - 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 Organochlorine Pesticides from Sediment by Gas Chromatography with Electron-Capture Detection*, Archives Environmental Contamination and Toxicology 47, 141-147 (2004)

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- 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)

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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	

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	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	

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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

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	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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