INDEPENDENT LABORATORY VALIDATION OF "RESIDUE ANALYTICAL METHOD FOR THE DETERMINATION OF RESIDUES OF BIFENTHRIN, CYPERMETHRIN, CYFLUTHRIN, DELTAMETHRIN, ESFENVALERATE, FENPROPATHRIN, LAMBDA-CYHALOTHRIN AND PERMETHRIN IN SEDIMENT"

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1.0 SUMMARY

The purpose of this study is to conduct an independent laboratory validation of the residue analytical method for bifenthrin, cypermethrin, cyfluthrin, deltamethrin, esfenvalerate, fenpropathrin, lambda-cyhalothrin and permethrin residues in sediment as described in the Morse Laboratories, LLC, Analytical Method (Reference 2), as written. The study is designed to demonstrate the utility, ruggedness, efficiency, and any inherent weakness in the subject method as written. This study was designed to fulfill the requirements of the U.S. EPA guidelines found in OCSPP 850.6100.

The study was conducted by ABC Laboratories of Columbia, Missouri, according to the protocol for ABC Study No. 68768, entitled, "Independent Laboratory Validation of "Residue Analytical Method for The Determination of Residues of Bifenthrin, Cypermethrin, Cyfluthrin, Deltamethrin, Esfenvalerate, Fenpropathrin, Lambda-Cyhalothrin and Permethrin in Sediment"". This report, ABC 68768, presents the data generated from bifenthrin, cypermethrin, cyfluthrin, deltamethrin, esfenvalerate, fenpropathrin, lambda-cyhalothrin and permethrin in sediment. The analyst involved with performing this ILV did not participate in the development of the original method.

The method under evaluation has a stated Limit of Quantitation (LOQ) of 0.10 ppb (0.1 µg/kg) for bifenthrin, cypermethrin, cyfluthrin, deltamethrin, esfenvalerate, fenpropathrin, and lambda-cyhalothrin, and 1.0 ppb (1.0 µg/kg) for permethrin. In this study, the method was validated on sediment at the LOQ and 10 x LOQ (0.10 ppb and 1.0 ppb for bifenthrin, cypermethrin, cyfluthrin, deltamethrin, esfenvalerate, fenpropathrin, and lambda-cyhalothrin and 1.0 ppb and 10.0 ppb for permethrin).

The first method validation trial was conducted on fresh water sediment from Minnesota with **no modifications to the method**. The validation included five replicates of control sample fortified with all the target compounds at their respective LOQs and 10x their LOQs. Trial 1 failed because the mean recoveries for all analytes at each fortification level not falling within 70% and 120%, and the presence of a peak in the control at the retention time of bifenthrin. The Sponsor Monitor indicated that it was preferable to have all analytes validated using a single sediment. As a result, a second trial was initiated.

The second method validation trial was conducted on fresh water sediment from Georgia with **no modifications to the method**. The validation included five replicates of control sample fortified with all the target compounds at their respective LOQs and 10x their LOQs.

A single analyst completed sample sets consisting of 13 samples in the course of 1 workday (8 hours) with GC/MSD analysis performed overnight, for a duration of 10 hours.

2.0 INTRODUCTION

The residue analytical method described in the referenced Morse method, entitled "Residue Analytical Method for The Determination of Residues of Bifenthrin, Cypermethrin, Cyfluthrin, Deltamethrin, Esfenvalerate, Fenpropathrin, Lambda-Cyhalothrin and Permethrin in Sediment" (Appendix 1) is applicable for the quantitation of determination of bifenthrin, cypermethrin, cyfluthrin, deltamethrin, esfenvalerate, fenpropathrin, lambda-cyhalothrin and permethrin in sediment. In this study, validation was performed on the representative matrix for which the method was designed, sediment.

Pyrethroid residues were extracted from a fortified 50-g sediment sample with 75 mL of methanol/water and 50 mL of hexane using a platform shaker for 60 minutes. The sample was centrifuged at ~4000 rpm for 5 minutes. A 10-mL aliquot of the hexane layer was taken and evaporated to dryness using a heating block set to ~40°C. Two milliliters of hexane were added to each sample and sonicated briefly. A Silica (Si) SPE column was preconditioned with 3 mL of hexane. The extract was then passed through the Si SPE column, where the analytes were retained and the eluate was allowed to go to waste. The Si SPE column was rinsed with 1 mL of hexane; the eluate was allowed to go to waste. The analytes were eluted by gravity flow into a glass tube using 6 mL 90:10 hexane:diethyl ether. The extracts were evaporated to dryness using a heating block set to ~40°C. The extracts were then reconstituted in 1 mL of 0.1% peanut oil in acetone and analyzed by GC/MSD. The limit of quantitation (LOQ) for all analytes in sediment was 0.10 ppb (µg/kg), except for permethrin at 1.0 ppb (µg/kg). The limit of detection (LOD) was estimated to be 0.03 ppb, except for permethrin at 0.3 ppb, or ~1/3 the LOQ.

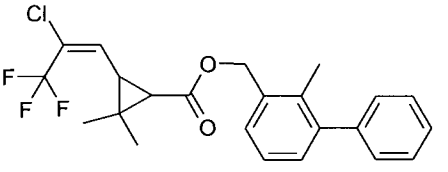
No communication, other than chromatography issues and recovery updates between the Sponsor Monitor and Study Director was required. The Sponsor Monitor (Pyrethroid Working Group) approved direct communication between the Study Director and the Method Developer in an appropriate area external to the laboratory to discuss and clarify the optimization procedures for the GC/MSD as they pertain to deltamethrin. The Method Developer was contacted after Method Trial 2 to specifically evaluate the results for lambda-cyhalothrin and bifenthrin as they compared to the results reported in the method. The Method Developer indicated that those results were similar to the method results.

3.0 MATERIALS AND METHODS

3.1 Test Substances

The reference analytical standards (test substances) used for this study were:

Bifenthrin

Common Name:	Bifenthrin
IUPAC Name:	2-methylbiphenyl-3-ylmethyl (*Z*)-(1*RS*,3*RS*)-3-(2-chloro-3-3-3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate
CAS Number:	82657-04-3
CAS Name:	(2-methyl[1,1'-biphenyl]-3-(2-chloro-3-3-3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate
Structural Formula:	

Supplier: FMC Corporation

Cypermethrin

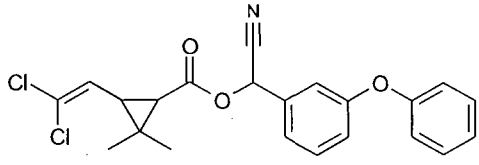
Common Name: Cypermethrin

IUPAC Name: (*RS*)-α-cyano-3-phenoxybenxyl (1*RS*,3*RS*;1*RS*,3*SR*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate

CAS Number: 52315-07-8

CAS Name: Cyano(3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate

Structural Formula:



Supplier: FMC Corporation (As separate cis- and trans- materials)

Cyfluthrin

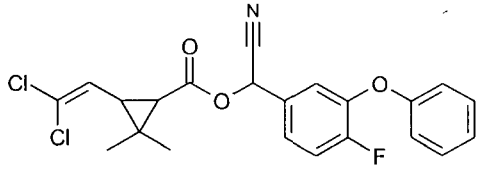
Common Name: Cyfluthrin

IUPAC Name: *(RS)*-α-cyano-4-fluoro-3-phenoxybenzyl (1*RS*,3*RS*;1*RS*,3*SR*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate

CAS Number: 68359-37-5

CAS Name: Cyano(4-fluoro-3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate

Structural Formula:



Supplier: Bayer CropScience

Deltamethrin

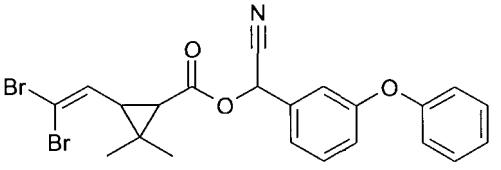
Common Name: Deltamethrin

IUPAC Name: (*S*)-α-cyano-3-phenoxybenzyl (1*R*,3*R*)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate

CAS Number: 52918-63-5

CAS Name: 1-[*R*-[1-α(*S**),3α]]-cyano(3-phenoxyphenyl)methyl 3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropanecarboxylate

Structural Formula:



Supplier: Bayer CropScience

Esfenvalerate

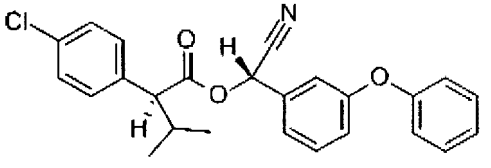
Common Name: Esfenvalerate

IUPAC Name: (*S*)-α-cyano-3-phenoxybenzyl (*S*)-2-(4-chlorophenyl)-3-methylbutyrate

CAS Number: 66230-04-4

CAS Name: [*S*-(*R**,*R**)]-cyano(3-phenoxyphenyl)methyl 4-chloro-2-(1-methylethyl)benzeneacetate

Structural Formula:



Supplier: DuPont Crop Protection

Fenpropathrin

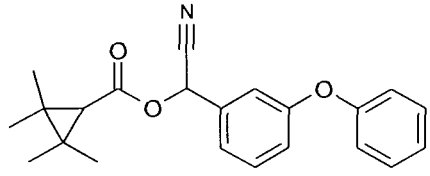
Common Name: Fenpropathrin

IUPAC Name: (*RS*)-α-cyano-3-phenoxybenzyl 2,2,3,3-tetramethylcyclopropanecarboxylate

CAS Number: 64257-84-7

CAS Name: Cyano(3-phenoxyphenyl)methyl 2,2,3,3-tetramethylcyclopropanecarboxylate

Structural Formula:



Supplier: Valent Corporation

Lambda-cyhalothrin

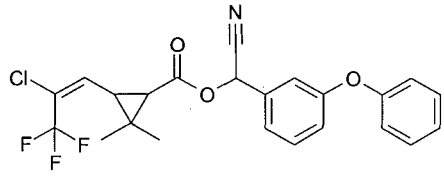
Common Name: Lambda-cyhalothrin

IUPAC Name: A 1:1 mixture of (*R*)-α-cyano-3-phenoxybenzyl (1*S*)-cis-3-(*Z*)-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate and (*S*)-α-cyano-3-phenoxybenzyl (1*R*)-cis-3-(*Z*)-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate

CAS Number: 91465-08-6

CAS Name: [1α(*S**),3α(*Z*)]-(±)-cyano(3-phenoxyphenyl)methyl 3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropanecarboxylate

Structural Formula:

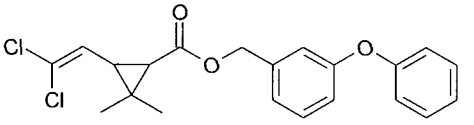


Supplier: Syngenta Crop Protection

Permethrin

Common Name:	Permethrin
IUPAC Name:	3-phenoxybenzyl (1*RS*,3*RS*;1*RS*,3*SR*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate
CAS Number:	52645-53-1
CAS Name:	(3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate

Structural Formula:



Supplier: FMC Corporation (As separate cis- and trans- materials)

Characterization and certification records for the analytical standards will be archived by the individual company members of the Pyrethroid Working Group (as indicated by the supplier designations listed above). The Certificates of Analysis are included in Appendix 2.

3.2 ***Test Systems***

In this study, the analytical method was validated on fresh water sediment.

Control sediment used in the study was obtained from Wabasha County, MN, for Sediment #507 (Trial 1), and Tift County, GA, for sediment #517 (Trial 2). The control sediment was stored refrigerated as part of this study until used for analysis. Storage temperatures were monitored on a daily basis and were typically at approximately 2-8°C.

The samples were assigned unique identification by the laboratory. Additional designations such as "control" and "fortified control," as appropriate, were also assigned by the laboratory.

3.3 *Equipment*

Equipment used is the same as that specified in the analytical method, except as follows:

EQUIPMENT DESCRIPTION	PRODUCT ID	SUPPLIER
Analytical Balance	Mettler XP205DR Analytical Balance Mettler BB2440 Analytical Balance	Mettler Instrument Corp (Hightstown, NJ)
Labware	Beckman GS 6S/HT benchtop centrifuge	Beckman Instruments (Palo Alto, CA)
	Supelco Visiprep 24-port vacuum box	Sigma-Aldrich (St. Louis, MO)
	250-mL PP bottles FisherBrand® Disposable 10 mL Pipettes FisherBrand® Disposable 15-mL glass culture tubes	Fisher Scientific (Pittsburgh, PA)
Pipettors	Gilson M100 (10-100µL) Gilson M250 (50-250µL) Gilson M1000 (1000µL)	Gilson (Middleton, WI)
Silica SPE	Silica (Si) cartridge	Agilent Technologies. (Santa Clara, CA)

3.4 *Reagents and Standards*

Reagents and standards used were of equivalent grade as that specified in the analytical method.

REAGENT	PRODUCT DESCRIPTION	VENDOR CATALOG NUMBER	SUPPLIER
Acetone	OPTIMA Grade	A929-4	Fisher Scientific (Fairlawn, NJ)
Water	HPLC Grade	W5-4	
Methanol	OPTIMA Grade	A454-4	
Hexane	OPTIMA Grade	H303-4	
Diethyl ether	HPLC Grade	309966-1L	Sigma-Aldrich (St. Louis, MO)
Peanut oil	LouAna Brand Peanut Oil, cooking grade	NA	Ventura Foods (Opelousas, LA)

3.5 *Principles of the Analytical Method*

The residue analytical method described in the referenced Morse method "Residue Analytical Method for The Determination of Residues of Bifenthrin, Cypermethrin, Cyfluthrin, Deltamethrin, Esfenvalerate, Fenpropathrin, Lambda-Cyhalothrin and Permethrin in Sediment"" was used for the analyses in this study. See Appendix 1 for the complete text of the methods as conducted at ABC Laboratories, Inc. The following is a summary of the method:

Pyrethroid residues were extracted from a fortified 50-g sediment sample with 75 mL of methanol/water and 50 mL of hexane using a platform shaker for 60 minutes. The sample was centrifuged at ~4000 rpm for 5 minutes. A 10-mL aliquot of the hexane layer was taken and evaporated to dryness using a heating block set to ~40°C. Two milliliters of hexane were added to each sample and sonicated briefly. A Silica (Si) SPE column was preconditioned with 3 mL of hexane. The extract was then passed through the Si SPE column, where the analytes were retained and the eluate was allowed to go to waste. The Si SPE column was rinsed with 1 mL of hexane; the eluate was allowed to go to waste. The analytes were eluted by gravity flow into a glass tube using 6 mL 90:10 hexane:diethyl ether. The extracts were evaporated to dryness using a heating block set to ~40°C. The extracts were then reconstituted in 1 mL of 0.1% peanut oil in acetone and analyzed by GC/MSD. The limit of quantitation (LOQ) for all analytes in sediment was 0.10 ppb (µg/kg), except for permethrin at 1.0 ppb (µg/kg). The limit of detection (LOD) was estimated to be 0.03 ppb, except for permethrin at 0.3 ppb, or ~1/3 the LOQ.

3.6 *Modifications, Interpretations, and Critical Steps*

The analytical method was performed exactly as written. The equipment and procedures used were run as written except for the following equivalencies:

Section 3.0 Instrumentation A HP Agilent 6890N chromatograph consisting of a HP 5973 MSD and an Agilent model 7683B injector. Agilent ChemStation software was used to collect the peak area responses while Excel was used to calculate the linear regression and to report the ppb found results.

Equivalent balances, centrifuge(s) and positive displacement pipettors were also used.

It was necessary to optimize the GC/MSD injection method to obtain the sensitivity needed for deltamethrin response. Instead of injecting 2 µL as indicated in the method, the injection volume was increased to 4 µL. A splitless injection mode (50mL/min, purge on at 2 min) was chosen instead of a pulsed splitless injection at 30psi for 1 min, purge flow to split vent 50 psi @ 2 min. The methane percentage to the detector was increased from 30% to 40%.

3.7 *Instrumentation*

The quantitative analysis of bifenthrin, cypermethrin, cyfluthrin, deltamethrin, esfenvalerate, fenpropathrin, lambda-cyhalothrin and permethrin was performed using an Agilent Model 7683B injector coupled to an Agilent Model 6890N GC/MSD system. The system parameters are shown in the tables below. Peak areas were used for quantitation.

GCMS Conditions:

System:	An integrated HP Agilent 6890N chromatograph consisting of a HP 5973 MSD and an Agilent model 7683B injector. The system is controlled and data processed by Agilent ChemStation Software
Column:	30 m × 0.25 mm i.d. fused silica column cross bonded with 0.25 µm film thickness Agilent CP-Sil 8CB-MS
Inlet liner:	4 mm i.d. double gooseneck splitless liner (unpacked)
Injection Volume:	4 µL
Injection mode:	Splitless, with purge flow to split vent 50 mL/min @ 2 minutes
Injection Temperature:	275 °C
Detector Transfer Line Temperature	280 °C
Flow Rate:	0.9 mL/min, constant flow
Carrier Gas:	Helium
Detector reagent gas:	Methane @ 40%
Dwell time:	50 msec
GC Conditions:	Initial: 80 °C, hold 1.00 minute Rate 1: 40 °C/minute to 180 °C Rate 2: 5 °C/minute Final: 305 °C
Retention Times: (Times are approximate)	

	Isomer Peak #	RT (min)
Bifenthrin	1	15.5
Fenpropathrin	1	15.8
Lambda-Cyhalothrin	1	16.9
	2	17.3
Permethrin	1	18.7
	2	19.0
Cyfluthrin	1	19.8
	2	20.0
	3	20.1
	4	20.2
Cypermethrin	1	20.4
	2	20.6
	3	20.7
	4	20.8
Esfenvalerate	1	22.0
	2	22.5
Deltamethrin	1	23.0
	2	23.5

Total Run Time:	~28.5 minutes

The detection method utilized a quadrupole GC/MS with negative ion CI (methane @ 40%) with a splitless injector and fused silica column. The acquisition method was adjusted to maximize the response of the fragment ions detected. The ion transitions for each analyte are shown in the table below:

Analyte	Target Ion (*m/z*)	Qualifier Ion 1 (*m/z*)	Qualifier Ion 2 (*m/z*)
Bifenthrin	386	387	241
Fenpropathrin	141	-	-
Lambda-Cyhalothrin	205	241	243
Permethrin	207	209	-
Cyfluthrin	207	209	171
Cypermethrin	207	209	171
Esfenvalerate	211	213	-
Deltamethrin	297	81	296

For each analytical run, a five-point standard curve was prepared by injecting constant volumes of standard solutions of a mixture of all analytes. Constant volume injections were used for sample extracts as well. A curve check standard was typically injected every 4 sample injections.

3.8 Calculations

Calculations were performed as directed by the method. ChemStation software was used to capture the peak areas for each compound of interest. Excel 2007 was used to create a standard curve based on linear regression. Linear regression was monitored to support the response linearity of the mass spectrometer detector. The regression functions were used to calculate a best fit line (from a set of standard concentrations in ng/mL versus peak response) to demonstrate that a linear relationship exists between analyte concentration and peak response, and that a response factor approach to calculation was appropriate.

The equation used for the least squares fit is:

$$y = mx + b$$

where:

y	=	peak response
x	=	ng/mL found for peak of interest
m	=	slope
b	=	y-intercept

Equations

The equations used for calculation purposes are as follows:

$$\text{Response Factor} = \frac{\text{analyte response (area) for standards}}{\text{concentration}}$$

$$\text{Average Response Factor} = \frac{\text{Sum of response factors of bracketing standards}}{2}$$

The amount of analyte (in µg/kg) found in the sample was calculated according to the following equation:

$$\mu g/kg = \frac{\text{total peak resp.}}{\text{avg. resp. fact.}} \times \frac{FV(mL)}{\text{Samp. wt.(g)}} \text{ x } \frac{\text{solv.(mL)}}{\text{aliq. (mL)}} \text{ x GC dil. factor}$$

Where:

total peak resp.	=	total of all peak responses (isomers) where applicable for sample.
avg. resp. fact.	=	average response factor for the bracketing standards
FV (mL)	=	volume of extract submitted to instrumentation (1.0 mL)
Samp. wt. (g)	=	amount of sample taken through the extraction process (50.0 g)
solv. (mL)	=	volume of extraction solvent added (50 mL)
aliq. (mL)	=	volume of extraction solvent taken through the method (10.0 mL)
GC dil. factor	=	dilution of sample extract required to produce analyte responses bracketed by standards (DF=1)

The percent recovery for fortified control samples was calculated as follows:

$$\%\text{Recovery} = \frac{\mu g/kg \text{ found in fortified control} - \mu g/kg \text{ found in control}}{\mu g/kg \text{ added}} x100$$

Example Calculations

All targeted analyte residues were calculated in an identical manner for both sediment types. Only examples of bifenthrin residue calculations in fresh water sediment will be provided and thus serve to illustrate the calculations for all other analytes in all sediment types.

1. Bifenthrin, Fresh Water Sediment, Set #2, 68768-014, **Control**:

   Peak response (area) = 9626

   $$\text{Response Factor Std 1} = \frac{148018}{5} = 29604$$

   $$\text{Response Factor Std 2} = \frac{308443}{10} = 30844$$

   $$\text{Average response factor} = \frac{29604 + 30844}{2} = 30224$$

   $$\mu\text{g/kg} = \frac{9626}{30224} \times \frac{1}{50} \text{ x } \frac{50}{5} \text{ x } 1$$

   μg/kg = 0.0318

   Reported = 0.03 μg/kg

2. Bifenthrin, Fresh Water Sediment, Set #2, 68768-015, **Control**

   Peak response (area) = 10693

   $$\text{Response Factor Std 1} = \frac{148018}{5} = 29604$$

   $$\text{Response Factor Std 2} = \frac{308443}{10} = 30844$$

   $$\text{Average response factor} = \frac{29604 + 30844}{2} = 30224$$

   $$\mu\text{g/kg} = \frac{10693}{30224} \times \frac{1}{50} \text{ x } \frac{50}{5} \text{ x } 1$$

   μg/kg = 0.0354

   Reported = 0.04 μg/kg

3. Bifenthrin, Fresh Water Sediment, Set #2, 68768-016, **Fortified Control**:

   Peak response (area) = 39956

   $$\text{Response Factor Std 1} = \frac{148018}{5} = 29604$$

$$\text{Response Factor Std 2} = \frac{308443}{10} = 30844$$

$$\text{Average response factor} = \frac{29604 + 30844}{2} = 30224$$

$$\mu\text{g/kg} = \frac{39956}{30224} \times \frac{1}{50} \text{ x } \frac{50}{5} \text{ x } 1$$

$$\mu\text{g/kg} = 0.1322$$

$$\text{Average } \mu\text{g/kg found in controls} = \frac{0.0318 + 0.0354}{2} = 0.0336$$

$$\frac{0.1322 - 0.0336}{0.10} \times 100 = 99\% \text{ Recovery}$$

1.0 SUMMARY

This report describes an analytical method used for the determination of residues of bifenthrin, cypermethrin, cyfluthrin, deltamethrin, esfenvalerate, fenpropathrin, lambda-cyhalothrin and permethrin in sediment samples. The method was developed by Syngenta Crop Protection, Inc. entitled "Residue Analytical Method for the Determination of Residues of Bifenthrin, Cypermethrin, Cyfluthrin, Deltamethrin, Esfenvalerate, Fenpropathrin, Lambda-Cyhalothrin and Permethrin in Sediment." A draft version of the method was used to generate the data. The results of this validation study, including any modifications needed to be made to the draft version are documented in the final version of the method found in Appendix 1.

Bifenthrin, cypermethrin, cyfluthrin, deltamethrin, esfenvalerate, fenpropathrin, lambda-cyhalothrin and permethrin residues were extracted from sediment by shaking with a methanol/water mixture and hexane for one hour. The sample was centrifuged and an aliquot of the upper hexane layer was evaporated to dryness and re-dissolved in a small volume of hexane. The hexane sample was then subjected to a silica solid phase extraction (SPE) cleanup step prior to residue determination by gas chromatography with mass selective detection using negative ion chemical ionization (GC-MS/NICI). The limit of quantitation of the method was 0.1 µg/kg for bifenthrin, cypermethrin, cyfluthrin, deltamethrin, esfenvalerate, fenpropathrin, lambda-cyhalothrin and 1.0 µg/kg for permethrin.

The purpose of this study was to validate this method according to the EPA Ecological Effects Test Guidelines: OPPTS 850.7100 Data Reporting for Environmental Chemistry Methods. A limit of quantitation (LOQ) of 0.1 µg/kg for all targeted analytes except permethrin, which was 1.0 µg/kg, and a working range from 0.1 µg/kg to 1.0 µg/kg for all targeted analytes except permethrin, which was from 1.0 µg/kg to 10 µg/kg, was validated in sediment. The validation was performed on two different types of sediment samples (fresh water and estuarine) collected from sites in California.

The study was conducted by Morse Laboratories, Inc. (Morse Labs) of Sacramento, California according to Protocol No. MLI-06-02, entitled "Validation of the Residue Analytical Method: "Residue Analytical Method for the Determination of Residues of Bifenthrin, Cypermethrin, Cyfluthrin, Deltamethrin, Esfenvalerate, Fenpropathrin, Lambda-Cyhalothrin and Permethrin in Sediment" and Protocol Amendments 1 and 2 (Appendix 2).

For each sediment type, the validation included five replicates of control sample fortified with all targeted compounds at their respective LOQs (0.1 µg/kg for all except permethrin, which was 1.0 µg/kg) and five replicates of control sample fortified with all targeted compounds at 10 times their respective LOQs (1.0 µg/kg for all except permethrin, which was 10 µg/kg). All fortified samples, along with two control samples and one reagent blank were analyzed for residues of all targeted compounds. The results, which demonstrate that the method is applicable for the determination of the targeted pyrethroid compounds in both types of sediment, are summarized below:

2.0 INTRODUCTION

Syngenta Crop Protection, Inc. method entitled "Residue Analytical Method for the Determination of Residues of Bifenthrin, Cypermethrin, Cyfluthrin, Deltamethrin, Esfenvalerate, Fenpropathrin, Lambda-Cyhalothrin and Permethrin in Sediment" was validated in this study. A draft version of the method was used to generate the data. The results of this validation study, including any modifications needed to be made to the draft version are documented in the final version of the method found in Appendix 1.

This study was conducted to satisfy guideline requirements described in the EPA Ecological Effects Test Guidelines: OPPTS 850.7100 Data Reporting for Environmental Chemistry Methods.

The method was validated on two types of sediment (fresh water and estarine) using unfortified and fortified samples of each. Fortifications were conducted at both the LOQ and 10 × LOQ of each targeted compound (5 replicates each). The validation results are reported herein.

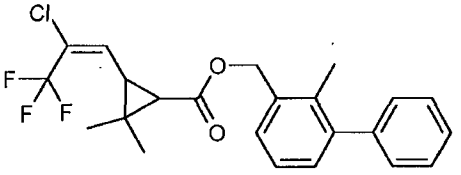
The study was conducted by Morse Laboratories, Inc. (Morse Labs) of Sacramento, California according to Protocol No. MLI-06-02, entitled "Validation of the Residue Analytical Method: "Residue Analytical Method for the Determination of Residues of Bifenthrin, Cypermethrin, Cyfluthrin, Deltamethrin, Esfenvalerate, Fenpropathrin, Lambda-Cyhalothrin and Permethrin in Sediment" and Protocol Amendments 1 and 2 (Appendix 2).

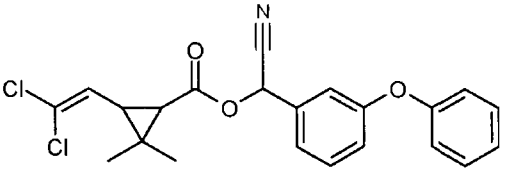
This report contains the following: test item and test system information, experimental details, method summary, calculations, results and discussion, example chromatography, and results generated from the analyses performed by Morse Laboratories, Inc.

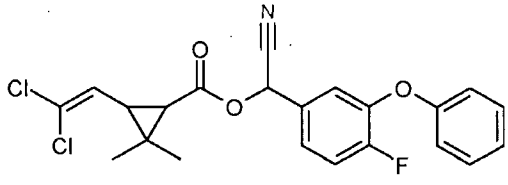
3.0 MATERIALS and METHODS

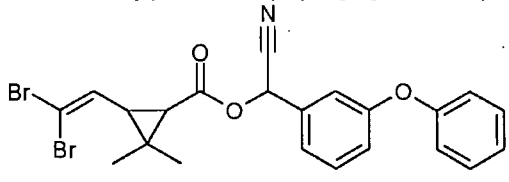
3.1 Test Items/Reference Substances

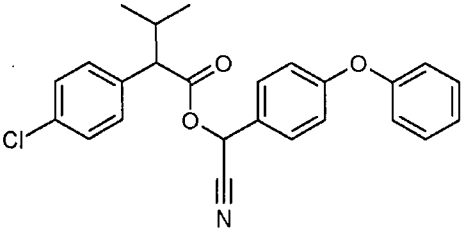
The analytical (reference) standards (test items) used in this study were:

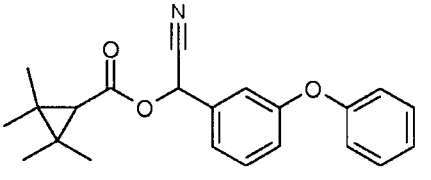
Compound	Bifenthrin
IUPAC Name:	2-methylbiphenyl-3-ylmethyl (*Z*)-(1*RS*,3*RS*)-3-(2-chloro-3-3-3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate
CAS Number:	82657-04-3
CAS Name:	(2-methyl[1,1'-biphenyl]-3-(2-chloro-3-3-3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate
Structure:	
% Purity:	97.8
Lot No.:	BI-29
Source:	FMC Agricultural Products
Expiration Date:	8/2007
Storage:	Typically -8 °C to -22 °C

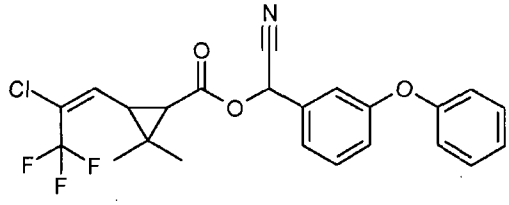
Compound	Cypermethrin
IUPAC Name:	(*RS*)-α-cyano-3-phenoxybenxyl (1*RS*,3*RS*;1*RS*,3*SR*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate
CAS Number:	52315-07-8
CAS Name:	Cyano(3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate
Structure:	
% Purity:	99.4
Lot No.:	AMS 202/102
Source:	Syngenta Crop Protection
Expiration Date:	3/2009
Storage:	Ambient

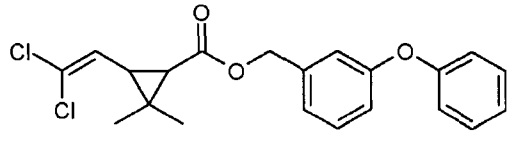
Compound	Cyfluthrin
IUPAC Name:	*(RS)*-α-cyano-4-fluoro-3-phenoxybenzyl (1*RS*,3*RS*;1*RS*,3*SR*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate
CAS Number:	68359-37-5
CAS Name:	Cyano(4-fluoro-3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate
Structure:	
% Purity:	50.2
Lot No.:	K-618
Source:	Bayer CropScience
Expiration Date:	12/31/13
Storage:	Typically -8 °C to -22 °C

Compound	Deltamethrin
IUPAC Name:	(*S*)-α-cyano-3-phenoxybenzyl (1*R*,3*R*)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate
CAS Number:	52918-63-5
CAS Name:	1-[*R*-[1-α(*S**),3α]]-cyano(3-phenoxyphenyl)methyl 3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropanecarboxylate
Structure:	
% Purity:	99.4
Lot No.:	K-1375
Source:	Bayer CropScience
Expiration Date:	9/8/10
Storage:	Typically -8 °C to -22 °C

Compound	Esfenvalerate
IUPAC Name:	(*S*)-α-cyano-3-phenoxybenzyl (*S*)-2-(4-chlorophenyl)-3-methylbutyrate
CAS Number:	66230-04-4
CAS Name:	[*S*-(*R**,*R**)]-cyano(3-phenoxyphenyl)methyl 4-chloro-2-(1-methylethyl)benzeneacetate
Structure:	
% Purity:	98.7
Lot No.:	YB656-058
Source:	DuPont Crop Protection
Expiration Date:	11/26/07
Storage:	Ambient

Compound	Fenpropathrin
IUPAC Name:	(*RS*)-α-cyano-3-phenoxybenzyl 2,2,3,3-tetramethylcyclopropanecarboxylate
CAS Number:	64257-84-7
CAS Name:	Cyano(3-phenoxyphenyl)methyl 2,2,3,3-tetramethylcyclopropanecarboxylate
Structure:	
% Purity:	99.7
Lot No.:	AS 459h
Source:	Valent
Expiration Date:	8/5/06
Storage:	Typically -8 °C to -22 °C

Compound	Lambda-cyhalothrin
IUPAC Name:	A reaction product containing equal quantities of (*S*)-α-cyano-3-phenoxybenzyl (*Z*)-(1*R*,3*R*)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate and (*R*)-α-cyano-3-phenoxybenzyl (*Z*)-(1*R*,3*R*)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate
CAS Number:	91465-08-6
CAS Name:	[1α(*S**),3α(*Z*)]-(±)-cyano(3-phenoxyphenyl)methyl 3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropanecarboxylate
Structure:	
% Purity:	98.7
Lot No.:	ASJ10012-04
Source:	Syngenta Crop Protection
Expiration Date:	8/2006
Storage:	Typically 1 °C to 8 °C

Compound	Permethrin
IUPAC Name:	3-phenoxybenzyl (1*RS*,3*RS*;1*RS*,3*SR*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate
CAS Number:	52645-53-1
CAS Name:	(3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate
Structure:	
% Purity:	99.7
Lot No.:	S01-2613
Source:	Syngenta Crop Protection
Expiration Date:	7/2006
Storage:	Typically -8 °C to -22 °C

Characterization data for the reference substances are maintained by the Pyrethroid Working Group.

The test and reference substances used in this study were procured and stored as directed on "Analytical Standard Certificate" or by the Study Monitor. All solutions made from the reference substances were stored according to the method.

3.2 Test Systems

Two types of sediment were evaluated in this study: fresh water and estuarine. Both control sediment samples were submitted to Morse Labs by Pacific Ecorisk of Matrinez, CA. The fresh water sediment (BUCGR) was received at the lab on March 30, 2006. The estuarine sediment (Paradise Cove) was received at the lab on May 12, 2006

Upon receipt of the samples at the laboratory, they were immediately placed in refrigerated storage (typically 1-8 °C), where they remained pending subsampling. The samples were transferred to freezer storage (typically -20 ± 5 °C) after subsampling.

3.3 Equipment and Reagents

The equipment and reagents used for the method validation were as outlined in the method (Section 2. Materials and Appendices 1 and 2). Identical or equivalent apparatus and materials were used, as permitted by the method.

3.4 Standard Solution Preparation

3.4.1 Stock Standard Solutions

The following concentrations of stock standard solutions were prepared. These solutions were prepared to contain each targeted analyte individually.

All targeted analytes except cyfluthrin:

Ten (10.0) mg (corrected for purity) of each applicable analytical standard were accurately weighed and quantitatively transferred to individual 50-mL volumetric flasks and brought to volume with acetone. The resulting concentration was 200 µg/mL.

Cyfluthrin:

Five (5.0) mg (corrected for purity) of cyfluthrin analytical standard were accurately weighed and quantitatively transferred to a 25-mL volumetric flask and brought to volume with acetone. The resulting concentration was 200 µg/mL.

3.4.2 Laboratory (Procedural) Fortification Standard Solutions

The following concentrations of fortification standard solutions were prepared. These solutions were prepared as mixtures containing all targeted analytes. The first concentration listed is for all analytes except permethrin, which is represented by the second concentration listed:

1.0 µg/mL/ 10 µg/mL:	125 µL of each of the 200 µg/mL stock standard solutions prepared for bifenthrin, cypermethrin, cyfluthrin, deltamethrin, esfenvalerate, fenpropathrin, and lambda-cyhalothrin and 1.25 mL of the 200 µg/mL stock standard solution prepared for permethrin were transferred to a 25-mL volumetric flask. The solution was brought to a final volume of 25 mL with acetone. The solution was mixed well.
0.1 µg/mL/ 1.0 µg/mL:	2.5 mL of the 1.0 µg/mL/10 µg/mL standard solution mixture prepared above were transferred to a 25-mL volumetric flask. The solution was brought to a final volume of 25 mL with acetone. The solution was mixed well.

3.4.3 Instrumentation (Calibration) Standard Solutions

The following concentrations of calibration standard solutions were prepared. These solutions were prepared as mixtures containing all targeted analytes. The first concentration listed is for all analytes except permethrin, which is represented by the second concentration listed:

20 ng/mL/ 200 ng/mL:	5.0 mL of a 0.1 µg/mL/1.0 µg/mL mixed fortification standard solution were transferred to a 25-mL volumetric flask. The contents were brought to volume with 0.1% (v/v) peanut oil in acetone. The solution was mixed well.
10 ng/mL/ 100 ng/mL:	2.5 mL of a 0.1 µg/mL/1.0 µg/mL mixed fortification standard solution were transferred to a 25-mL volumetric flask. The contents were brought to volume with 0.1% (v/v) peanut oil in acetone. The solution was mixed well.
5.0 ng/mL/ 50 ng/mL:	1.25 mL of a 0.1 µg/mL/1.0 µg/mL mixed fortification standard solution were transferred to a 25-mL volumetric flask. The contents were brought to volume with 0.1% (v/v) peanut oil in acetone. The solution was mixed well.

2.5 ng/mL/ 25 ng/mL:	625 µL of a 0.1 µg/mL/1.0 µg/mL mixed fortification standard solution were transferred to a 25-mL volumetric flask. The contents were brought to volume with 0.1% (v/v) peanut oil in acetone. The solution was mixed well.
1.0 ng/mL/ 10 ng/mL:	250 µL of a 0.1 µg/mL/1.0 µg/mL mixed fortification standard solution were transferred to a 25-mL volumetric flask. The contents were brought to volume with 0.1% (v/v) peanut oil in acetone. The solution was mixed well.
0.5 ng/mL/ 5.0 ng/mL:	125 µL of a 0.1 µg/mL/1.0 µg/mL mixed fortification standard solution were transferred to a 25-mL volumetric flask. The contents were brought to volume with 0.1% (v/v) peanut oil in acetone. The solution was mixed well.

3.5 Sample Preparation

Each untreated control sample was processed for analysis by transferring the entire sample received into a large metal bowl. Any rocks or plant debris present was removed by hand. The samples were then mixed well using a large metal spoon. Following processing, the samples were divided into six equal portions and each portion was placed into a properly labeled plastic bag. The bagged portions were placed into frozen storage (typically at less than or equal to -15 °C) where they remained until removal for subsampling and analysis.

3.6 Fortification Procedures

Each validation set per sediment type consisted of one reagent blank, two control samples, five control samples fortified at the LOQ (0.1 µg/kg for all analytes except permethrin which was 1.0 µg/kg) for each analyte and five control samples fortified at 10 × LOQ for each analyte as shown below:

Matrix	Sample Type	Fortifying Compounds	Fortification Level	# of Samples
None	Reagent blank	None	0.0 µg/kg	1
Sediment	Control	None	0.0 µg/kg	2
Sediment	Spike	All targeted analytes (except Permethrin) and Permethrin	0.1 µg/kg (LOQ) 1.0 µg/kg (LOQ)	5
Sediment	Spike	All targeted analytes (except Permethrin) and Permethrin	1.0 µg/kg (10 x LOQ) 10 µg/kg (10 x LOQ)	5

Twelve 50.0-g portions of a specific sediment type control sample were used as samples for each validation set. Samples were designated as controls or fortified controls. Fortified controls were each fortified with all eight analytes at either the LOQ (50 µL of a mixed standard solution containing 0.1 µg/mL of all analytes except permethrin which was at 1.0 µg/mL) or 10 × LOQ (50 µL of a mixed standard solution containing 1.0 µg/mL of all analytes except permethrin which was at 10 µg/mL). Once fortified, the fortified control samples along with appropriate controls and a reagent blank, were analyzed as per method.

3.7 Calibration Procedures

Determination and quantitation of pyrethroid residues were conducted using gas chromatography employing negative chemical ionization mass selective detection (GC-MS/NICI). The instrument calibration standards for this study were prepared by making appropriate dilutions of stock standard solutions of the test item. The standard solutions bracketed the working range. The lowest standard of the working range was no less than half the concentration of the lowest sample concentration expected.

Calibration standards were injected concurrently with the sample injections during the course of the analytical run. For preparation of the calibration curve, the standards were injected sequentially during the run. Because calculations were based on bracketing standard response factors, appropriate standards were injected so that no more than four sample injections were bracketed between standards. Each analytical run began and ended with a standard injection.

3.8 Analytical Method

The method developed by Syngenta Crop Protection, Inc. entitled "Residue Analytical Method for the Determination of Residues of Bifenthrin, Cypermethrin, Cyfluthrin, Deltamethrin, Esfenvalerate, Fenpropathrin, Lambda-Cyhalothrin and Permethrin in Sediment" was used for the analyses in this study. A draft version of the method was used to generate the data. The results of this validation study, including any modifications needed to be made to the draft version are documented in the final version of the method found in Appendix 1.

Bifenthrin, cypermethrin, cyfluthrin, deltamethrin, esfenvalerate, fenpropathrin, lambda-cyhalothrin and permethrin residues were extracted from sediment by shaking with a methanol/water mixture and hexane for one hour. The sample was centrifuged and an aliquot of the upper hexane layer was evaporated to dryness and re-dissolved in a small volume of hexane. The hexane sample was then subjected to a silica solid phase extraction (SPE) cleanup step prior to residue determination by gas chromatography with mass selective detection using negative ion chemical ionization (GC-MS/NICI). The limit of quantitation of the method was 0.1 µg/kg for bifenthrin, cypermethrin, cyfluthrin, deltamethrin, esfenvalerate, fenpropathrin, lambda-cyhalothrin and 1.0 µg/kg for permethrin.

3.9 Instrumentation

The GC conditions employed for the analyses in this study were as follows:

Operating Conditions

Instrument:	An Agilent 6890 gas chromatograph equipped with an Agilent 5973N mass selective detector operated in negative chemical ionization mode, a HP 7683 autosampler, and a HP G1701CA MS ChemStation.
Column:	30 m × 0.25 mm i.d. fused silica column crossbonded with 0.25 µm film thickness Varian CP-Sil 8CB-MS
Inlet liner:	4 mm i.d. double gooseneck splitless liner (unpacked)
Injection volume:	2 µL
Injection mode:	pulsed splitless, 30 psi for 1 minute, purge flow to split vent 50 mL/min @ 2 minutes
Carrier gas:	Helium
Column flow:	0.9 mL/min, constant flow

Detector reagent gas: Methane @ 30%

Dwell time: 50 msec

Tuning: Prior to analysis, the instrument is autotuned for ions m/z 185, 351 and 449

Temperatures: Injector: 275 °C

GC/MSD Transfer line: 280 °C

Column:
Initial: 80 °C, hold 1.00 minute
Rate 1: 40 °C/minute to 180 °C
Rate 2: 5 °C/minute
Final: 305 °C

Ions monitored:

Analyte	Target Ion (*m/z*)	Qualifier Ion 1 (*m/z*)	Qualifier Ion 2 (*m/z*)
Bifenthrin	386	387	241
Fenpropathrin	141	-	-
Lambda-Cyhalothrin	205	241	243
Permethrin	207	209	-
Cyfluthrin	207	209	171
Cypermethrin	207	209	171
Esfenvalerate	211	213	-
Deltamethrin	297	81	296

Retention times:

Analyte	Peak #	Retention time (min)
Bifenthrin	1	18.1
Fenpropathrin	1	18.5
Lambda-Cyhalothrin	1	19.6
	2	19.9
Permethrin	1	21.5
	2	21.8
Cyfluthrin	1	22.5
	2	22.7
	3	22.8
	4	22.9
Cypermethrin	1	23.2
	2	23.4
	3	23.5
	4	23.6
Esfenvalerate	1	24.9
	2	25.3
Deltamethrin	1	25.9
	2	26.3

3.10 Calculations

A validated software application was used to generate a standard curve based on linear regression. The regression functions were used to calculate a best-fit line and to demonstrate linearity of the GC/MSD detector system.

Calculations for instrumental analysis were conducted using an Excel spreadsheet. Average response factors for bracketing standards were used to determine sample residues. Where compounds produced multiple peaks (isomers), the total area under all peaks was used in the calculations.

The equations used for calculation purposes are as follows:

1. $$Response\ factor = \frac{analyte\ response\ (area)\ for\ standard}{concentration}$$

2. $$Average\ response\ factor = \frac{Sum\ of\ response\ factors\ of\ bracketing\ standards}{2}$$

3. The amount of analyte (in µg/kg) found in the sample was calculated according to the following equation:

$$\mu g/kg = \frac{total\ peak\ resp.}{avg.\ resp.\ fact.} \times \frac{FV\ (mL)}{Samp.\ wt.\ (g)} \times \frac{solv.\ (mL)}{aliq.\ (mL)} \times GC\ dil.\ factor$$

where:

total peak resp.	=	total of all peak responses (isomers) where applicable for sample.
avg. resp. fact.	=	average response factor for the bracking standards
FV (mL)	=	volume of extract submitted to instrumentation (1.0 mL)
Samp. wt. (g)	=	amount of sample taken through the extraction process (50.0 g)
solv. (mL)	=	volume of extraction solvent added (50 mL)
aliq. (mL)	=	volume of extraction solvent taken through the method (10.0 mL)
GC dil. factor	=	dilution of sample extract required to produce analyte responses bracketed by standards

4. The percent recovery for fortified control samples was calculated as follows:

$$\%\ Recovery = \frac{\mu g/kg\ found\ in\ fortified\ control - \mu g/kg\ found\ in\ control}{\mu g/kg\ added} x\,100$$

Example Calculations

All targeted analyte residues were calculated in an identical manner for both sediment types. Only examples of bifenthrin residue calculations in estuarine sediment will be provided and thus serve to illustrate the calculations for all other analytes in all sediment types.

1. ML ticket #83942, Bifenthrin, Estuarine Sediment, Set #3, Paradise Cove, **Control** 5 (Figure 10):

Peak response (area) = 3071

1. $Response\ factor\ std.1 = \frac{6282}{1.0} = 6282$

   $Response\ factor\ std.2 = \frac{121315}{20} = 6066$

2. $Average\ response\ factor = \frac{6282 + 6066}{2} = 6174$

3. $\mu g/kg = \frac{3071}{6174} \times \frac{1.0\,mL}{50.0\,g} \times \frac{50\,mL}{10.0\,mL} \times 1$

   $\mu g/kg = 0.049740849$

   $Reported = <0.1\ \mu g/kg$

2. ML ticket #83942, Bifenthrin, Estuarine Sediment, Set #3, Paradise Cove, **Fortified Control** 21 @ 0.1 µg/kg (Figure 15):

   Peak response (area) = 8465

1. $Response\ factor\ std.1 = \frac{6312}{1.0} = 6312$

   $Response\ factor\ std.2 = \frac{5926}{1.0} = 5926$

2. $Average\ response\ factor = \frac{6312 + 5926}{2} = 6119$

3. $\mu g/kg = \frac{8465}{6119} \times \frac{1.0\,mL}{50.0\,g} \times \frac{50\,mL}{10.0\,mL} \times 1$

   $\mu g/kg = 0.138339598$

   $Reported = 0.138\ \mu g/kg$

4. $\%\ Recovery = \frac{0.138\,\mu g/kg - 0.0469\,\mu g/kg}{0.1\,\mu g/kg\ added} x\,100$

   $Recovery = 91\%$

1. Introduction and Summary

1.1 Scope

The analytical procedure described is suitable for the determination of residues of bifenthrin, cypermethrin, cyfluthrin, deltamethrin, esfenvalerate, fenpropathrin, lambda-cyhalothrin and permethrin (Figures 1-8) in sediment using an external standardisation procedure. The limit of quantitation of the method is 0.1 $\mu g\ kg^{-1}$ for bifenthrin, cypermethrin, cyfluthrin, deltamethrin, esfenvalerate, fenpropathrin, lambda-cyhalothrin and 1.0 $\mu g\ kg^{-1}$ for permethrin.

Figure 1

Compound : Bifenthrin

IUPAC Name : 2-methylbiphenyl-3-ylmethyl (*Z*)-(1*RS*,3*RS*)-3-(2-chloro-3-3-3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate

CAS Number : 82657-04-3

CAS Name : (2-methyl[1,1'-biphenyl]-3-(2-chloro-3-3-3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate

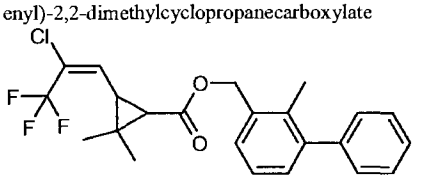


Figure 2

Compound : Cypermethrin

IUPAC Name : (*RS*)-α-cyano-3-phenoxybenxyl (1*RS*,3*RS*;1*RS*,3*SR*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate

CAS Number : 52315-07-8

CAS Name : Cyano(3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate

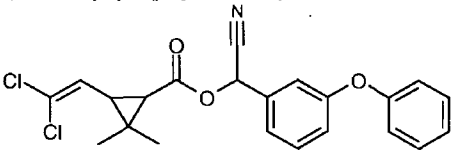


Figure 3

Compound	:	Cyfluthrin
IUPAC Name	:	*RS*)-α-cyano-4-fluoro-3-phenoxybenzyl (1*RS*,3*RS*;1*RS*,3*SR*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate
CAS Number	:	68359-37-5
CAS Name	:	Cyano(4-fluoro-3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate

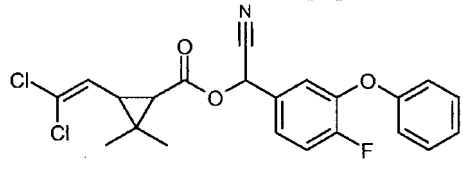


Figure 4

Compound	:	Deltamethrin
IUPAC Name	:	(*S*)-α-cyano-3-phenoxybenzyl (1*R*,3*R*)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate
CAS Number	:	52918-63-5
CAS Name	:	1-[*R*-[1-α(*S**),3α]]-cyano(3-phenoxyphenyl)methyl 3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropanecarboxylate

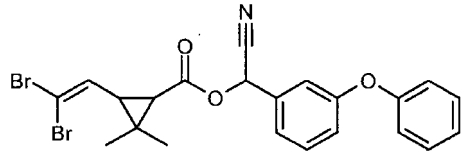


Figure 5

Compound	:	Esfenvalerate
IUPAC Name	:	(*S*)-α-cyano-3-phenoxybenzyl (*S*)-2-(4-chlorophenyl)-3-methylbutyrate
CAS Number	:	66230-04-4
CAS Name	:	[*S*-(*R**,*R**)]-cyano(3-phenoxyphenyl)methyl 4-chloro-2-(1-methylethyl)benzeneacetate

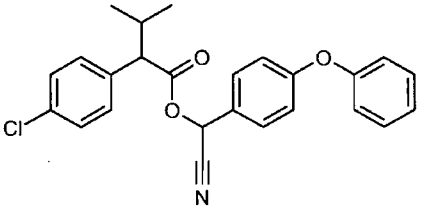


Figure 6

Compound	:	Fenpropathrin
IUPAC Name	:	(*RS*)-α-cyano-3-phenoxybenzyl 2,2,3,3-tetramethylcyclopropanecarboxylate
CAS Number	:	64257-84-7
CAS Name	:	Cyano(3-phenoxyphenyl)methyl 2,2,3,3-tetramethylcyclopropanecarboxylate

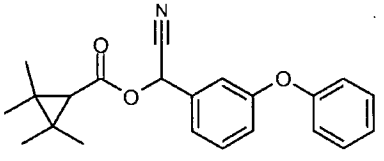


Figure 7

Compound : Lambda-cyhalothrin

IUPAC Name : A reaction product containing equal quantities of (*S*)-α-cyano-3-phenoxybenzyl (*Z*)-(1*R*,3*R*)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate and (*R*)-α-cyano-3-phenoxybenzyl (*Z*)-(1*R*,3*R*)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate

CAS Number : 91465-08-6

CAS Name : [1α(*S**),3α(*Z*)]-(±)-cyano(3-phenoxyphenyl)methyl 3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropanecarboxylate

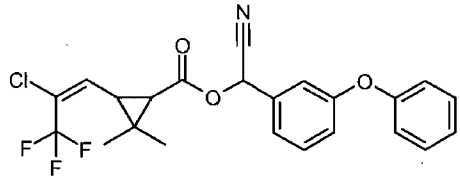


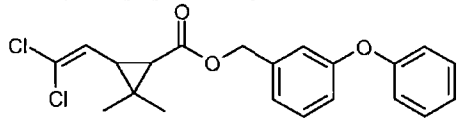
Figure 8

Compound : Permethrin

IUPAC Name : 3-phenoxybenzyl (1*RS*,3*RS*;1*RS*,3*SR*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate

CAS Number : 52645-53-1

CAS Name : (3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate



1.2 Method Summary

Bifenthrin, cypermethrin, cyfluthrin, deltamethrin, esfenvalerate, fenpropathrin, lambda-cyhalothrin and permethrin residues are extracted from sediment by shaking with methanol/water mixture and hexane for one hour. The sample is centrifuged and an aliquot of the upper hexane layer evaporated to dryness and re-dissolved in a small volume of hexane. The hexane sample is then subjected to a silica solid phase extraction (SPE) procedure prior to residue determination by gas

chromatography with mass selective detection using negative ion chemical ionisation (GC-MS/NICI). The limit of quantitation of the method is 0.1 $\mu g\ kg^{-1}$ for bifenthrin, cypermethrin, cyfluthrin, deltamethrin, esfenvalerate, fenpropathrin, lambda-cyhalothrin and 1.0 $\mu g\ kg^{-1}$ for permethrin.

2. Materials

The recommended equipment and reagents are described in Appendices 1 and 2. Equipment with equivalent performance specifications and reagents of comparable purity can be substituted provided that they can be shown to be suitable.

2.1 Apparatus

See Appendix 1 for a list of apparatus used during this method.

2.2 Reagents

All solvents and other reagents must be of high purity, i.e. pesticide grade solvents and analytical grade reagents. Extreme care must be taken to avoid contamination of the reagents used. See Appendix 2 for a list of reagents used in this method.

2.3 Preparation of Analytical Standards

It is recommended that the following precautions should be taken when weighing the analytical materials.

1. Ensure good ventilation.
2. Wear gloves and laboratory coat.
3. Prevent inhalation and contact with mouth.
4. Wash any contaminated area immediately.

Weigh out accurately, using a 5 figure balance, sufficient bifenthrin, cypermethrin, cyfluthrin, deltamethrin, esfenvalerate, fenpropathrin, lambda-cyhalothrin and permethrin analytical standards to allow dilution in acetone to give a 200 $\mu g\ mL^{-1}$ stock solutions in a volumetric flasks. Make serial dilutions of these stock solutions to give 100 $\mu g\ mL^{-1}$, 10 $\mu g\ mL^{-1}$, 1.0 $\mu g\ mL^{-1}$, 0.1 $\mu g\ mL^{-1}$ and 0.01 $\mu g\ mL^{-1}$ standards in acetone. If required, mixed standards can also be prepared to 0.01 $\mu g\ mL^{-1}$ in acetone.

When not in use, always store the standard solutions in a refrigerator at ≤7°C to prevent decomposition and/or concentration of the standard. Analytical standards should be replaced with freshly prepared standards after six months.

2.4 Safety Precautions and Hazards

The following information is included as an indication to the analyst of the nature and hazards of the reagents used in this procedure. If in any doubt, consult a monograph such as 'Hazards in the Chemical Laboratory', Edited by S G Luxon, The Chemical Society, London (Reference 1).

Reagent Hazards

	Acetone	Hexane	Methanol	Diethyl Ether
Harmful Vapour	✓	✓	✓	✓
Highly Flammable	✓	✓	✓	✓
Harmful by Skin Absorption	✗	✓	✗	✗
OES Short Term (mg m^{-3})	3560	3600	310	1500

In all cases avoid breathing vapour. Avoid contact with eyes and skin.

2.5 Time Required for Analysis

The methodology is normally performed with a batch of 20 samples over the course of 1 day.

2.6 Work Stoppages

The analytical procedure can be stopped at various points for overnight and weekend breaks except where specified in the analytical procedure. Acceptable external standard recoveries will validate the work stoppages. Samples should be stored in sealed vessels at a temperature of ≤7°C.

3. Analytical Procedure

3.1 Sample Preparation

Sediment samples should be thoroughly mixed prior to taking an aliquot for analysis to ensure sample homogeneity.

3.2 Extraction

a) Weigh a representative amount of aquatic sediment (50 g) into a plastic screw capped centrifuge bottle (250 ml size). At least one untreated control and two control samples fortified with known amounts of bifenthrin, cypermethrin, cyfluthrin, esfenvalerate, fenpropathrin, lambda-cyhalothrin and permethrin in acetone should be analysed alongside each batch of samples to demonstrate acceptable performance of the method and allow recovery corrections to be made if desired.

Note : To avoid sample cross contamination, extraction vessels should be used only once and discarded after use.

b) Add methanol/water solution [1:1 v/v, 75 mL] and hexane (50 mL) to the sample and shake the sample using a mechanical shaker for 60 minutes.

c) Centrifuge the sample at a speed that disperses any emulsions formed on shaking e.g. 4000 rpm for five minutes.

3.3 Solid Phase Extraction

a) Transfer an aliquot of the upper hexane layer from the sediment extract equivalent to 10 g of sediment (10 mL) into a test tube (10 mL size). Evaporate the sample to dryness under a stream of clean, dry air in a heating block set to 40°C. Re-dissolve the sample in hexane (2 mL), with ultrasonication.

b) Place a Varian Silica Bond Elut™ solid phase extraction cartridge (500 mg, 3 mL size) on a suitable vacuum manifold. Add hexane (3 mL) and draw through under vacuum to the level of the top frit at a rate of approximately 2 mL min^{-1}, discarding the eluate.

c) Transfer the sample aliquot from section 3.3 (a) onto the cartridge and allow to percolate through under gravity or low vacuum (approx. 200 mbar). Discard the eluate.

d) Add hexane (1 mL) to the cartridge and allow to percolate through under gravity or low vacuum. Discard the eluate.

e) Place a collection tube (10 mL) in the manifold rack. Elute the analytes from the column with hexane/diethyl ether solution [9:1 v/v, 6 mL] drawing through under gravity or low vacuum at a rate of approximately 2 mL min^{-1}, collecting the eluate in the tube.

f) Evaporate the column eluate to dryness under a stream of clean, dry air in a heating block with the temperature set to 40 °C. Re-dissolve the sample in acetone

+ 0.1% (v/v) peanut oil solution (1 mL) with ultrasonication. Transfer the sample to an autosampler vial ready for final determination by GC-MS/NICI.

Note : The 0.1% peanut oil in acetone solution is used to minimise the effect of matrix related GC-MSD response enhancement and to minimise possible peak tailing due to adsorption.

3.4 Preparation of GC-MSD Calibration Standards

GC-MSD calibration standards should be prepared in acetone + 0.1% (v/v) peanut oil solution. The 0.1% peanut oil in acetone solution is used to minimise the effect of matrix related GC-MSD response enhancement and to minimise possible peak tailing due to adsorption.

For example, to prepare a 1.0 ng mL^{-1} calibration standard, transfer 1 mL of a 0.01 µg mL^{-1} bifenthrin, cypermethrin, cyfluthrin, deltamethrin, esfenvalerate, fenpropathrin, lambda-cyhalothrin and permethrin mixed standard in acetone to a volumetric flask (10 mL) and dilute to 10 mL volume with acetone.

4. Final Determination by GC-MSD

The following instrumentation and conditions have been found to be suitable for this analysis in this laboratory. Other instrumentation can also be used, however optimisation may be required to achieve the desired separation and sensitivity. The operating manuals for the instruments should always be consulted to ensure safe and optimum use.

Instrument Conditions

GC system	:	Agilent 6890 with split/splitless injector
MSD system	:	Agilent 5973 with negative ion chemical ionization
Injection temperature	:	275°C
Injection liner	:	4 mm i.d. double gooseneck splitless liner (unpacked)
Column	:	Varian CPSil 8 30 m × 0.25 mm, 0.25 µm film thickness (5% diphenyl, 95% dimethylpolysiloxane)
Column flow rate	:	0.9 mL min^{-1} constant flow
Injection mode	:	Pulsed splitless, 30 psi for 1 min, purge flow to split vent 50 psi @2 min
Injection volume	:	2 µL
Column temperature program	:	80°C for 1 min then program at 40°C/min to 180°C, hold for 0 min then program at 5 °C/min to 305 °C, hold for 0 min.

MS transfer line temp	:	280°C
Ionization mode	:	Negative ion CI
Reagent gas	:	Methane
System calibration	:	Autotune
System resolution	:	Low
Acquisition type	:	Selected Ion Monitoring (SIM)

	Target Ion	Qualifier 1	Qualifier 2
Bifenthrin	*m/z* = 386	*m/z* = 387	*m/z* = 241
Fenpropathrin	*m/z* = 141	-	-
Lambda-cyhalothrin	*m/z* = 205	*m/z* = 241	*m/z* = 243
Permethrin	*m/z* = 207	*m/z* = 209	-
Cyfluthrin	*m/z* = 207	*m/z* = 209	*m/z* = 171
Cypermethrin	*m/z* = 207	*m/z* = 209	*m/z* = 171
Esfenvalerate	*m/z* = 211	*m/z* = 213	-
Deltamethrin	*m/z* = 297	*m/z* = 81	*m/z* = 296

Retention Times

Compound Name	Peak	Retention Time (min)
Bifenthrin	1	18.1
Fenpropathrin	1	18.5
Lambda-cyhalothrin	1 2	19.6 19.9
Permethrin	1 2	21.5 21.8
Cyfluthrin	1 2 3 4	22.5 22.7 22.8 22.9
Cypermethrin	1 2 3 4	23.2 23.4 23.5 23.6
Esfenvalerate	1 2	24.9 25.3
Deltamethrin	1 2	25.9 26.3

Typical chromatograms are shown in Appendix 4.

5. Calculation of Results

Residues may be calculated using an external standardisation procedure. For lambda-cyhalothrin, permethrin, cyfluthrin, cypermethrin, esfenvalerate and deltamethrin, isomer peaks are resolved in the GC chromatogram. The peak areas of the individual isomer peaks for each pyrethroid should be summed together and a total residue value calculated for each compound.

5.1 Using Mean Bracketed Single-Point Calibration

a) Make repeated injections of a standard containing bifenthrin, cypermethrin, cyfluthrin, deltamethrin, esfenvalerate, fenpropathrin, lambda-cyhalothrin and permethrin at an appropriate concentration into the GC-MSD operated under conditions as described in Section 4. When a consistent response is obtained, measure the peak areas obtained for each peak.

b) Make an injection of each sample solution and measure the peak areas of the peaks corresponding to the each analyte.

c) Re-inject the standard solution after a maximum of four injections of sample solutions.

d) Calculate the residues in the sample, expressed as $\mu g\ kg^{-1}$, using a mean standard response from each of the injections bracketing the sample as follows.

$$\text{Residue} = \frac{\text{PK area (SA)}}{\text{PK area (STD)}} \times \frac{\text{Standard Conc.}}{\text{Sample Conc.}}$$

PK area (SA) = Peak response for sample
PK area (STD) = Average peak response for bracketing standards
Standard Conc. = Concentration of standard (ng mL^{-1})
Sample Conc. = Sample concentration (g mL^{-1})

If residues need to be corrected for average percentage recovery, then the equation below should be used.

$$\text{Corrected Residue} = \frac{\text{Residue} \times 100}{\text{Average percentage Recovery}} (\mu g\ kg^{-1})$$

When the average percentage recovery is greater than 100%, the sample residue values should not be corrected.

5.2 Using Multi-Point Calibration

Bifenthrin, cypermethrin, cyfluthrin, deltamethrin, esfenvalerate, fenpropathrin, lambda-cyhalothrin and permethrin residues may be calculated in $\mu g\ kg^{-1}$ for each sample as follows.

a) Prepare standard solutions containing bifenthrin, cypermethrin, cyfluthrin, deltamethrin, esfenvalerate, fenpropathrin, lambda-cyhalothrin and permethrin over a concentration range appropriate to the expected residues in the samples (for example, 50% LOQ to 10x LOQ). An appropriate number of different concentrations within this range should be prepared (at least four). Injections of these standard solutions should be interspersed throughout the analysis, after a maximum of four injections of sample solutions.

b) Make an injection of each standard and sample solution into the GC-MSD operated under conditions as described in Section 4 and measure the peak areas of the peaks corresponding to each analyte.

c) Generate calibration curve parameters using an appropriate regression package.

d) The following equation can be rearranged and used to calculate residues as follows:

$$y = mx + c$$

Where y is the instrument response value, x is the standard concentration, m is the gradient of the line of best fit ("X-variable 1" in MS Excel) and c is the intercept value. An example of this equation generated using the experimental values of m and c should be included in the raw data, as should the "R-Square" value for the regression.

Re-arrangement for x gives

$$x = \frac{y - c}{m}$$

e) Alternatively (depending on the regression analysis software available) a quadratic equation may be used to fit the data. In this case the following general equation should be re-arranged and used to calculate residues:

$$y = a + bx + cx^2$$

Where y is the instrument response value, x is the standard concentration and a, b, c are constants.

g) Calculate the residues in the sample, expressed as μg kg^{-1}, as follows

$$\text{Residue } (\mu g\ kg^{-1}) = \frac{\text{Analyte found } (ng\ mL^{-1})}{\text{Sample conc. } (g\ mL^{-1})}$$

Where analyte found (ng mL^{-1}) is calculated from the standard calibration curve and sample conc. is the final sample concentration in g mL^{-1}.

If residues need to be corrected for average percentage recovery, then the equation below should be used.

$$\text{Corrected Residue} = \frac{\text{Residue} \times 100}{\text{Average percentage Recovery}}\ (\mu g\ kg^{-1})$$

When the average percentage recovery is greater than 100%, the sample residue values should not be corrected.

6. Control and Recovery Experiments

Control experiments should be completed as Section 3 for each set of samples analysed to verify that samples are free from bifenthrin, cypermethrin, cyfluthrin, deltamethrin, esfenvalerate, fenpropathrin, lambda-cyhalothrin and permethrin contamination. A minimum of one control should be analysed with each batch of samples.

At least two recovery experiments (untreated samples accurately fortified with a known amount of bifenthrin, cypermethrin, cyfluthrin, deltamethrin, esfenvalerate, fenpropathrin, lambda-cyhalothrin and permethrin prior to extraction) should also be completed alongside each batch of samples. Provided the recovery values are acceptable they may be used to correct any residues found. The recovery levels should be appropriate to the residue levels expected.

Recovery data is generally considered acceptable when the mean values are between 70% and 120% and with a relative standard deviation of ≤20%.

7. Interference

7.1 Matrix

Due to the high selectivity of the detection technique, no interference arising from the sediment matrix has been observed.

7.2 *Reagent and Solvent Interference*

Using high purity solvents and reagents no interference has been found, however it is recommended that each batch of reagents or solvent is checked for contamination prior to use.

7.3 Labware Interference

The method uses disposable labware which minimises the possibility of cross contamination. All labware should be discarded after use and not re-used.

Appendix 1 : Apparatus

General laboratory glassware e.g. volumetric flasks, available from Fisher Scientific, Liberty Lane, Hampton, NH 03842, USA.

250 ml screw capped polypropylene centrifuge bottles, available from Fisher Scientific, Liberty Lane, Hampton, NH 03842, USA.

10 mL disposable glass test tubes, available from Fisher Scientific, Liberty Lane, Hampton, NH 03842, USA

Varian Bond Elut™ Silica solid phase extraction columns 500 mg, 3 mL size, available from Varian Inc. 24021 Frampton Avenue, Harbor City, CA 90710, USA.

SPE sample processing station, available from Varian Inc. 24021 Frampton Avenue, Harbor City, CA 90710, USA.

Gas chromatograph fitted with a mass selective detector e.g. Agilent 6890 GC fitted with a 5973 series mass selective detector, available from Agilent Technologies, 395 Page Mill Road, Palo Alto, CA 94304 USA.

CP SIL-8 CB Low bleed MS capillary column 30 m × 0.25 mm id, 0.25 µm film thickness, available from Varian Inc. 24021 Frampton Avenue, Harbor City, CA 90710, USA.

Double gooseneck injection liner for Agilent splitless injectors (4 mm i.d.), available from Restek Corporation, 110 Benner Circle, Bellefonte, PA 168230-8812, USA.

Crimp cap autosampler vials and caps available from Fisher Scientific, Liberty Lane, Hampton, NH 03842, USA.

Appendix 2 : Reagents

All solvents and other reagents must be of high purity, e.g. glass distilled/HPLC grade solvents and analytical grade reagents. Particular care must be taken to avoid contamination of the reagents used.

Hexane, methanol, acetone and diethyl ether, pesticide grade, available from B & J Brand Solvents, from Scientific Products Division of Baxter Healthcare Corporation, USA (Tel: 312-689-8410).

Peanut oil, cooking grade, available from Planters Company, East Hanover, NJ 07936.

Bifenthrin, cypermethrin, cyfluthrin, deltamethrin, esfenvalerate, fenpropathrin, lambda-cyhalothrin and permethrin analytical standards, available from Dr. Ehrenstorfer GmbH, Bgm.-Schlosser-Str. 6 A, 86199 Augsburg, Germany.