USGS
science for a changing world

Methods of Analysis—Determination of Pyrethroid Insecticides in Water and Sediment Using Gas Chromatography/Mass Spectrometry

Techniques and Methods 5–C2

U.S. Department of the Interior
U.S. Geological Survey

Methods of Analysis—Determination of Pyrethroid Insecticides in Water and Sediment Using Gas Chromatography/ Mass Spectrometry

By Michelle L. Hladik, Kelly L. Smalling, and Kathryn M. Kuivila

Techniques and Methods 5–C2

U.S. Department of the Interior
U.S. Geological Survey

U.S. Department of the Interior
KEN SALAZAR, Secretary

U.S. Geological Survey
Suzette M. Kimball, Acting Director

U.S. Geological Survey, Reston, Virginia: 2009

Suggested reference:
Hladik, M.L., Smalling, K.L., and Kuivila, K.M., 2009, Methods of analysis—Determination of pyrethroid insecticides in water and sediment using gas chromatography/mass spectrometry: U.S. Geological Survey Techniques and Methods 5–C2, 18 p.

Contents

Figures

Tables

Conversion Factors

SI to Inch/Pound

Multiply	By	To obtain
Length		
centimeter (cm)	0.3937	inch (in.)
micrometer (µm)	3.937×10^{-5}	inch (in.)
millimeter (mm)	0.03937	inch (in.)
meter (m)	39.37	inch (in.)
Volume		
liter (L)	0.2642	gallon (gal)
microliter (µL)	2.642×10^{-7}	gallon (gal)
milliliter (mL)	0.000264	gallon (gal)
mL/min	0.0338	ounce per minute
Mass		
gram (g)	0.03527	ounce avoirdupois (oz)
kilogram (kg)	2.205	pound avoirdupois (lb)
microgram (µg)	3.527×10^{-8}	ounce avoirdupois (oz)
milligram (mg)	3.527×10^{-5}	ounce avoirdupois (oz)
nanogram (ng)	3.527×10^{-11}	ounce avoirdupois (oz)
Pressure		
kilopascal (kPa)	0.1450	pound-force per inch (lbf/in)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:

$$°F = (1.8 \times °C) + 32$$

Abbreviated water-quality units of measurement used in this report:

Å	angstrom
cm	centimeter
g	gram
L	liter
L/min	liter per minute
m	meter
mg	milligram
mg/mL	milligram per milliliter
mL	milliliter
mL/min	milliliter per minute
mm	millimeter
ng	nanogram
ng/L	nanogram per liter
ng/µL	nanogram per microliter
nm	nanometer

µA	microampere
µg/kg	microgram per kilogram
µg/mL	microgram per milliliter
µL	microliter
µm	micrometer (micron)

Other abbreviations used in this report

(additional information or clarification given in parentheses)

ACS	American Chemical Society
amu	atomic mass unit
ASTM	American Society for Testing and Materials
C	sample concentration (equations 3 and 5)
CAS	Chemical Abstracts Service (American Chemical Society)
CCV	continuing calibration verification
COB	carryover blank
DCM	dichloromethane
DF	dilution factor
E	extract concentration (equation 2)
EI	electron ionization
EtOAc	ethyl acetate
GC	gas chromatograph
GC/ECD	gas chromatography with electron capture detection
GC/MS	gas chromatography with mass spectrometry
GC/MS/MS	gas chromatography with tandem mass spectrometry
GC/NCIMS	gas chromatography with negative-chemical-ionization mass spectrometry
GF/F	glass-fiber filter (grade GF/F)
GPC	gel-permeation chromatography
HLB	hydrophilic-lipophilic balance
HPLC	high-performance liquid chromatography
i.d.	inner diameter
ISTD	internal standard
LOD	limits of detection
MAE	microwave-assisted extraction
MDL	method detection limit (text and equation 7)
MeOH	methanol
min	minute
MRL	minimum reporting level
MS	mass spectrometer
MS/MS	tandem mass spectrometry
m/*z*	mass-to-charge ratio
n	number of samples
nd	not detected
NWIS	National Water Information System (USGS)
PFRG	Pesticide Fate Research Group (USGS)

PFTBA	perfluorotributylamine
PPE	personal protective equipment
psi	pound per square inch
QA/QC	quality assurance and quality control
QC	quality control
RF	response factor (equation 1)
RPD	relative percent difference (equation 7)
RSD	relative standard deviation
RT	retention time
s	second
SD	standard deviation
SIS	selected ion storage
SOP	standard operating procedure
SPE	solid-phase extraction
SRM	standard reference material
SSC	suspended-solids concentration
TAP	time at parameter
USEPA	U.S. Environmental Protection Agency
USGS	U.S. Geological Survey
UV	ultraviolet
UV-Vis	ultraviolet and visible light
v/v	volume-to-volume
W_s	sediment extracted (equation 4)

This page intentionally left blank.

Methods of Analysis—Determination of Pyrethroid Insecticides in Water and Sediment Using Gas Chromatography/Mass Spectrometry

By Michelle L. Hladik, Kelly L. Smalling, and Kathryn M. Kuivila

Abstract

A method for the determination of 14 pyrethroid insecticides in environmental water and sediment samples is described. The method was developed by the U.S. Geological Survey in response to increasing concern over the effects of pyrethroids on aquatic organisms. The pyrethroids included in this method are ones that are applied to many agricultural and urban areas.

Filtered water samples are extracted for pyrethroids using solid-phase extraction (SPE) with no additional cleanup steps. Sediment and soil samples are extracted using a microwave-assisted extraction system, and the pyrethroids of interest are separated from co-extracted matrix interferences by passing the extracts through stacked graphitized carbon and alumina SPE cartridges, along with the use of high-performance liquid chromatography and gel-permeation chromatography (HPLC/GPC). Quantification of the pyrethroids from the extracted water and sediment samples is done using gas chromatography with mass spectrometry (GC/MS) or gas chromatography with tandem mass spectrometry (GC/MS/MS).

Recoveries in test water samples fortified at 10 ng/L ranged from 83 to 107 percent, and recoveries in test sediment samples fortified at 10 µg/kg ranged from 82 to 101 percent; relative standard deviations ranged from 5 to 9 percent in the water samples and 3 to 9 percent in the sediment samples. Method detection limits (MDLs), calculated using U.S. Environmental Protection Agency procedures (40 CFR 136, Appendix B), in water ranged from 2.0 to 6.0 ng/L using GC/MS and 0.5 to 1.0 ng/L using GC/MS/MS. For sediment, the MDLs ranged from 1.0 to 2.6 µg/kg dry weight using GC/MS and 0.2 to 0.5 µg/kg dry weight using GC/MS/MS. The matrix-spike recoveries for each compound, when averaged for 12 environmental water samples, ranged from 84 to 96 percent, and when averaged for 27 environmental sediment samples, ranged from 88 to 100 percent.

Introduction

Pyrethroid use as an insecticide has been increasing in recent years as a replacement for organophosphate insecticides that are being phased out because of water-quality concerns (California Department of Pesticide Regulation, 2005). Pyrethroids are used in both agricultural and urban (commercial and residential) areas. The occurrence of pyrethroids is of concern because pyrethroids are known to be highly toxic to aquatic organisms, especially those that are sediment-dwelling (Hill, 1989). Because of their hydrophobicity (Laskowski, 2002), pyrethroids tend to sorb to particulate matter present in natural waters and are typically detected in sediments. As pyrethroid use continues to increase in both urban and agricultural settings, it is important to have robust, sensitive methods that are capable of measuring these compounds at environmentally relevant concentrations (below acute toxicity levels) in both water and sediment. These methods will also help scientists understand pyrethroid behavior in the environment.

Multiple methods exist to measure pyrethroids at environmentally relevant concentrations (below acute toxicity), including those already developed by the U.S. Geological Survey's (USGS) Pesticide Fate Research Group for water (Hladik and others, 2008) and sediment (Smalling and Kuivila, 2008). Other published methods to measure pyrethroids include liquid-liquid extraction of whole (unfiltered) water (Bonwick and others 1995; Fernandez-Guiterrez and others, 1998; Lee and others, 2002) or the measurement of just the dissolved fraction (filtered water) using solid-phase extraction (SPE) cartridges (C8, C18, or HLB) (van der Hoff and others, 1996; Hengel and others, 1997; Lee and others, 2002). However, because pyrethroids tend to loosely associate with the walls of sample containers, especially when a sample is pumped slowly through an SPE cartridge, a bottle wash with an organic solvent must be included in the extraction step. Many methods have been developed to extract organic compounds effectively from sediments; they typically include three main steps: (1) extraction using either sonication or pressurized fluid extraction techniques (You and Lydy, 2004; Smalling and Kuivila, 2008), (2) matrix removal by prepacked SPE cartridges or packed columns with Florisil or silica gel (Esteve-Turrillas and others, 2004; LeBlanc and others, 2004; Smalling and others 2007), and (3) sulfur removal by activated copper or gel-permeation chromatography (Houtman and others, 2007; Sanchez-Burnete and others, 2002). A good, sensitive method for pyrethroid analysis in sediments must include an effective cleanup step that removes greater than 90 percent of the background matrix. This will not only decrease method detection limits, but it will improve analysis by tandem GC/MS/MS.

Following extraction and separation, the detection and quantification of pyrethroids is performed by using gas chromatography with electron capture detection (GC/ECD) (You and others, 2004), gas chromatography with mass spectrometry (GC/MS) (Hladik and others, 2008; Smalling and Kuivila, 2008), and gas chromatography with negative-chemical-ionization mass spectrometry (GC/NCIMS) (Bonwick and others, 1995). Typical method detection limits have ranged from 1 to 10 ng/L in water and from 1 to 5 µg/kg in sediment using GC/MS; however, lower MDLs can be obtained using GC/ECD (0.5–1 µg/kg) (You and others, 2004).

The method presented is for the analysis of 14 pyrethroids in filtered water and sediment (and soil) matrices. The extraction of pyrethroids from 1-L filtered water samples is achieved with SPE. Extraction of sediment is achieved with microwave-assisted extraction, and the removal of co-extracted matrix interferences is achieved by stacked SPE and HPLC/GPC. Quantification of pyrethroids in all matrices is done with GC/MS and GC/MS/MS. These methods had acceptable recoveries (greater than 70 percent) for all matrices tested. MDLs in water ranged from 2.0 to 6.0 ng/L for GC/MS and 0.5 to 1.0 ng/L for GC/MS/MS. For sediment, the MDLs ranged from 1.0 to 2.6 µg/kg dry weight for GC/MS and 0.2 to 0.5 µg/kg dry weight for GC/MS/MS.

Purpose and Scope

The purpose of this report is to describe a method for the extraction and quantification of pyrethroids from water and sediment samples. The methods described in this report were developed by the USGS's Pesticide Fate Research Group (PFRG), Sacramento, California, to analyze 14 pyrethroids. Water samples were extracted using SPE. Sediment samples were extracted by microwave-assisted extraction (MAE), with SPE and HPLC-GPC cleanup of matrix interferences that occur in sediment extracts. Quantitation for both extracts was achieved with GC/MS and GC/MS/MS. This report also provides extraction recoveries along with relative standard deviations, method detection limits, and matrix-spike recoveries for a set of environmental samples.

The method of analysis for water samples described in this report is assigned a USGS method number O-2143-09, USGS method code GM011, and PFRG code PYRWAT. The method of analysis for sediment samples described in this report is assigned a USGS method number O-6143-09, USGS method code GM012 (for suspended sediments) and GM013 (for bed sediments), and PFRG code PYRSED. These unique codes represent the method of analysis as it is described in the report and can be used to identify the method. This method provides an effective option to environmental scientists seeking pyrethroid analyses for samples of water and sediment, with minimal contamination bias, relatively low MDLs, good recoveries, and excellent precision. The method will contribute to the improved understanding of the occurrence, fate, and transport of pyrethroid insecticides in the environment.

Analytical Method

Organic Compounds and Parameter Codes: Pyrethroid insecticides in filtered water using SPE and GC/MS and GC/MS/MS—USGS method number O-2143-09, USGS method code GM011, and PFRG code PYRWAT. Pyrethroid insecticides in bed sediments, suspended sediments, and soils using MAE, SPE, HPLC-GPC, and GC/MS and GC/MS/MS—USGS method number O-6143-09, USGS method codes GM012 and GM013, and PFRG code PYRSED.

1. Scope and Application

This method is suitable for determining the pyrethroids listed in *table 1* (the structure of each compound is shown in *fig. 1*), at nanogram-per-liter concentrations in water samples and at microgram-per-kilogram concentrations in sediment samples. Method compounds, Chemical Abstracts Service (CAS) numbers, molecular weights, and parameter codes are listed in *table 1*.

Table 1. CAS number, molecular weight, and U.S. Geological Survey parameter codes for each pyrethroid.

[This report contains Chemical Abstracts Service Registry Numbers (CASRN), which is a Registered Trademark of the American Chemical Society. Chemical Abstracts Service (CAS) recommends the verification of the CASRNs through CAS Client Services. The five-digit parameter codes are used by the U.S. Geological Survey to uniquely identify a specific constituent or property in the National Water Information System (NWIS) database. amu, atomic mass unit]

Pyrethroid	CAS number	Molecular weight (amu)	Water parameter code	Bed-sediment parameter code	Suspended-sediment parameter code	Soil parameter code
Allethrin	584-79-2	302.41	66586	66588	66587	67541
Bifenthrin	82657-04-3	422.87	65067	64151	63415	67545
Cyfluthrin	68359-37-5	434.27	65074	65109	65122	67569
λ-Cyhalothrin	91456-08-6	449.86	65086	64162	65134	67674
Cypermethrin	52315-07-8	416.30	65075	64156	65123	67571
Deltamethrin	52918-63-5	505.24	65077	65110	65125	67581
Esfenvalerate	66230-04-4	419.91	65081	64159	65129	67601
Fenpropathrin	39515-41-8	349.42	65083	65111	65131	67631
τ-Fluvalinate	102851-06-9	502.93	65106	65114	65148	67727
Permethrin	52645-53-1	391.29	65099	64168	65143	67695
Resmethrin	10453-86-8	338.45	65104	65113	65147	67723
Sumithrin (phenothrin)	26002-80-2	350.46	65100	65112	65144	67697
Tefluthrin	79538-32-2	418.74	67731	67733	67732	67734
Tetramethrin	7696-12-0	331.41	66657	66659	66658	67738

2. Method Summary

2.1 Water Samples

Water samples are collected in the field into 1-L amber glass bottles using the methods outlined by Hladik and others (2009), U.S. Geological Survey (2006), and Ward and Harr (1990). Samples are chilled immediately, shipped to the PFRG, and refrigerated at 4 °C until analysis (within 48 hours of collection). Samples are filtered either in the field or in the laboratory using a GF/F-grade glass-fiber filter (GF/F). The water samples are spiked with surrogate standards and passed through an HLB SPE cartridge. Adsorbed compounds are eluted from the cartridge with ethyl acetate (EtOAc). The empty sample bottle is rinsed with dichloromethane (DCM). Both the eluent and the bottle rinsate are evaporated in a hood using a gentle stream of nitrogen to 0.2 mL; internal standards are then added. The concentrations of the pyrethroids in the extracts are determined by gas chromatography/mass spectrometry (GC/MS) or gas chromatography/tandem mass spectrometry (GC/MS/MS).

2.2 Sediment Samples

Sediment or soil samples are collected in the field using the methods such as those outlined by Radtke (2005), typically into 500-mL amber glass jars. Samples are chilled immediately, shipped to the PFRG, and frozen at –20 °C until analysis (within 6 months). For extraction, the samples are thawed, and the percentage moisture is adjusted to 50 percent. The samples are extracted with MAE two times using DCM:methanol. The extract is reducted to 0.5 mL using a TurboVap II system. Co-extracted matrix interferences are removed by loading samples onto stacked graphitized carbon and alumina SPE cartridges. The pyrethroids are eluted from the SPE cartridge with DCM. The eluent is exchanged into EtOAc. Further removal of sulfur and matrix is achieved by high-performance liquid chromatography with gel-permeation chromatography (HPLC-GPC) of the EtOAc fraction.

The GPC eluent is evaporated in a hood using a gentle stream of nitrogen to a final volume of 0.2 mL. The concentrations of the pyrethroids in the extracts are determined by gas chromatography/mass spectrometry (GC/MS) or gas chromatography with tandem mass spectrometry (GC/MS/MS).

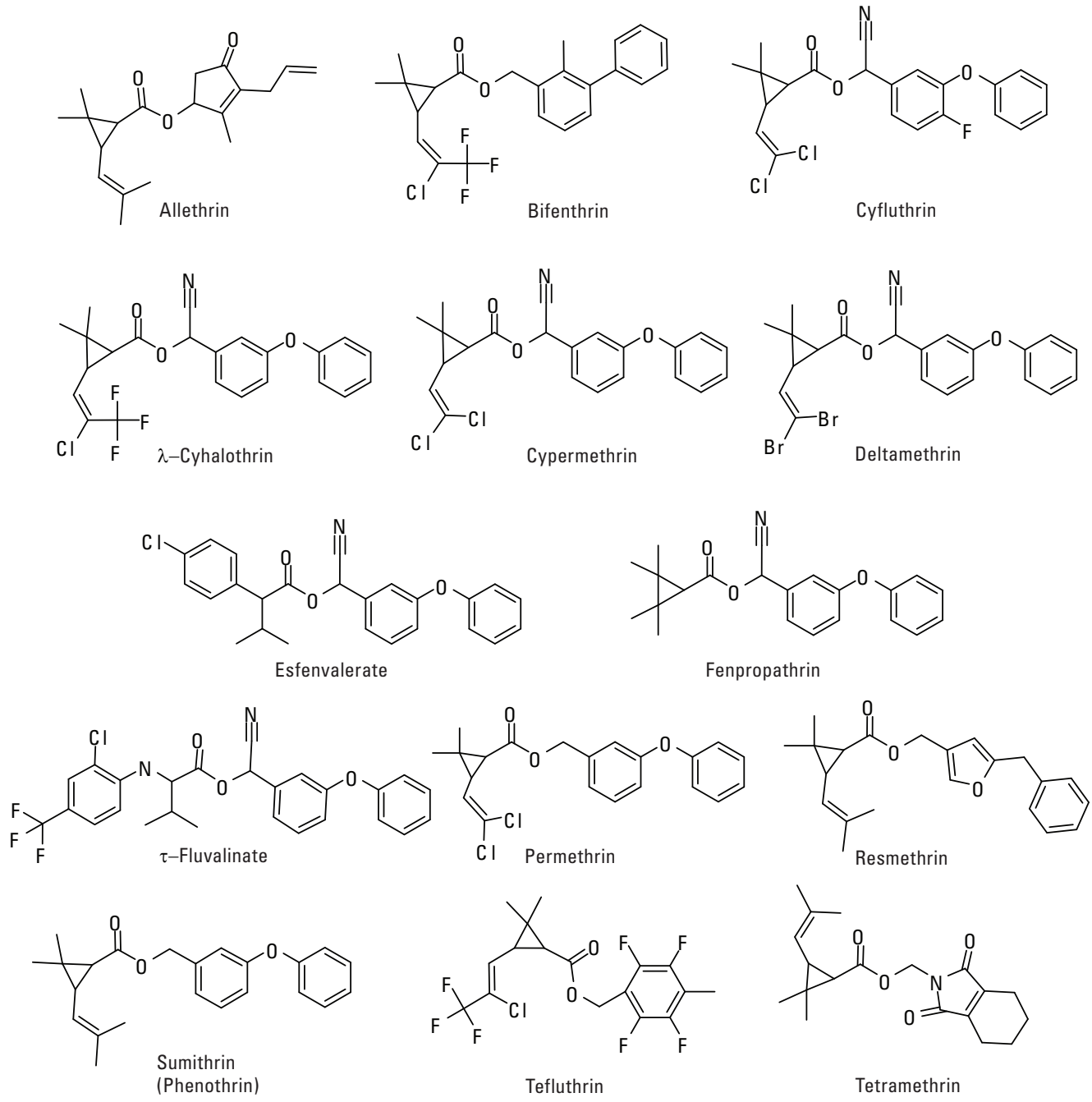

Figure 1. Chemical structures of the 14 pyrethroids included in the methods.

3. Safety Precautions and Waste Disposal

The following safety precautions are followed:

3.1 All steps that use organic solvents are performed in a well-vented fume hood.

3.2 The microwave exhaust and TurboVap exhaust must be vented to a fume hood.

3.3 Appropriate personal protective equipment (PPE) is used during the handling of reagents and chemicals.

3.4 Disposable nitrile gloves do not provide adequate protection from DCM. Polyvinyl acetate gloves will provide adequate protection. Alternatively, the analyst may wear double nitrile gloves, and if DCM comes in contact with the nitrile gloves, the gloves must be removed immediately.

3.5 Precautions are taken when handling the gas chromatograph (GC) injector or working with the mass spectrometer (MS), as temperatures in their heated zones can be near 300° C. These areas must be allowed to cool before touching them.

3.6 All liquid waste (other than the water that passes through the SPE cartridges) produced during the extraction is considered "organic waste" and must be placed in thick-walled carboys and disposed of according to local regulations. The solid-waste stream produced during sample analysis comprises SPE cartridges, extracted sediment or soil, sodium sulfate, and assorted disposable glassware (such as glass pipettes and GC vials). Once the solid-waste items have been dried in a hood (until no organic solvent remains), they can be disposed of according to local policy.

4. Interferences

Compounds that compete with or displace the compounds of interest from the SPE cartridge materials might cause interferences or low method recoveries. In addition, humic and fulvic acids might influence extraction efficiency, and because some samples might have a complex nature, pyrethroid recoveries may be reduced. Possible interferences are addressed with matrix-spiked samples and surrogate compounds.

The purpose of representative sampling is to characterize the true concentrations of pyrethroid insecticides in environmental samples. Pyrethroid insecticides are a common ingredient in household pesticide products. Field and laboratory personnel should be aware of this and limit their exposure to these products prior to sample collection or sample handling. The potential for contamination bias during sample collection or handling is monitored by the use of field blanks and laboratory blanks.

5. Apparatus and Instrumentation

The following apparatus and instrumentation are used:

5.1 *Analytical balances*—Balances for sediment samples capable of accurately weighing 5.00 g ± 0.01 g. Balance for standard preparation accurately weighs 5.000 mg ±0.001 mg.

5.2 *Microwave-assisted extractor*—CEM MSP 1000 MAE (Matthews, North Carolina), including pre-cleaned Teflon extraction vessels.

5.3 *Filtration pump*—Stainless-steel filter plate with 1 m of 9.5-mm i.d. Teflon tubing capable of pumping water at a flow rate of 0.5 L/min attached to a MasterFlex peristaltic pump (Cole Parmer, Vernon Hills, Illinois).

5.4 *TurboVap*—Zymark Corporation (Hopkinton, Massachusetts) TurboVapII Concentration Workstation, including precleaned glass tubes (0.2 to 1.0 mL graduated).

5.5 *N-evap*—Organomation Associates, Inc. (Berlin, Massachusetts) N-EVAP Nitrogen Evaporator and 12-mL glass concentrator tubes.

5.6 *SPE vacuum manifold*—Includes vial rack to hold 12-mL glass concentrator tubes.

5.7 *SPE cartridges*—Oasis HLB cartridges (6 cc, 500 mg, Waters, Milford, Massachusetts) for water samples; Carboprep 90 graphitized carbon cartridges (6 cc, 500 mg, Restek, Bellefonte, Pennsylvania) stacked on top of Sep-Pak Alumina A cartridges (500 mg, Waters, Milford, Massachusetts) for sediment samples.

5.8 *HPLC-GPC benchtop system*—Scientific Systems Inc. (State College, Pennsylvania) Series I isocratic HPLC pump and unltraviolet-visible (UV-Vis) detector (set to 254 nm) with a PL-gel guard column (10 µm) and PL-gel analytical column (10 µm, 50 Å, 300 × 7.5 mm; Polymer Laboratories, Walnut Creek, California).

5.9 *GC/MS benchtop system*—Varian CP-3800 gas chromatograph coupled to a Saturn 2000 ion-trap mass spectrometer with Varian Workstation software *v 6.4* and Combi-Pal autosampler (Walnut Creek, California).

5.10 *GC/MS analytical column*—DB-5ms (30 m × 0.25 mm × 0.25 µm; Agilent Technologies, Palo Alto, California).

5.11 Pre-cleaned glassware including pipettes, microsyringes, concentrator tubes, funnels, and graduated cylinders—Everything but the micro syringes are baked at 450 °C for a minimum of 4 hours.

6. Reagents and Consumable Materials

6.1 *Analytical standards*—Neat solutions of pyrethroids (allethrin, bifenthrin, cyfluthrin, λ-cyhalothrin, cypermethrin, deltamethrin, esfenvalerate, fenpropathrin, τ-fluvalinate, permethrin, resmethrin, sumithrin [phenothrin], tefluthrin, tetramethrin; Chem Service, West Chester, Pennsylvania).

6.2 *Internal standard (ISTD) solution*—Neat solutions of the ISTDs, d_{10}-acenaphthene, d_{10}-phenanthrene, and d_{10}-pyrene (Cambridge Isotope Laboratories, Andover, Massachusetts).

6.3 *Surrogate standard solution*—The surrogate, phenoxy-$^{13}C_6$-*cis*-permethrin at 50 µg/mL (Cambridge Isotope Laboratories, Andover, Massachusetts).

6.4 *Deionized water*—Generated by purification of tap water to American Society of Testing and Materials (ASTM) Type II, or better, water (Picosystem Plus, Hydro Service and Supplies, Inc., Durham, North Carolina).

6.5 *Solvents*—DCM, methanol (MeOH), EtOAc; all Thermo Fisher Scientific (Pittsburg, Pennsylvania) Optima grade or better.

6.6 *Sodium sulfate*—Anhydrous, 10/60 mesh, American Chemical Society (ACS)-certified (Thermo Fisher Scientific, Pittsburgh, Pennsylvania), baked at 450 °C for a minimum of 4 hours.

6.7 *Helium carrier gas (99.999 percent)*—Gas chromatograph carrier gas.

6.8 *Nitrogen gas (99.999 percent)*—For evaporation of organic solvent.

6.9 *Carbon dioxide gas (99.9999 percent)*—For drying SPE cartridges.

6.11 *Glass-fiber filters*—142-mm diameter, 0.7-µm nominal pore size, GF/F-grade glass-fiber filters (Whatman, Piscataway, New Jersey) prebaked at 450 °C for a minimum of 4 hours.

7. Standards Preparation Procedure

7.1 *Primary standard solutions*—Individual stock solutions of 1.0 mg/mL for each pyrethroid, surrogate, and ISTD are prepared by accurately weighing, to the nearest 0.0001 g, 4–5 mg of the pure material into a 7-mL amber glass vial. Add 1 mL of acetone (using a micro-syringe) per mg of the weighed compound.

7.2 *Pyrethroid standard stock solution*—Stock solution containing 40 ng/µL of each pyrethroid is prepared by diluting individual 1-mg/mL solutions (1.0 mL each) into EtOAc in a 25-mL volumetric flask.

7.3 *Internal standard stock solution*—Stock solution containing 10 ng/µL of ISTD is prepared by diluting 1 mL of each 1-mg/mL solutions into EtOAc in a 100-mL volumetric flask.

7.4 *Pyrethroid standard solution*—Solution containing 10 ng/µL of pyrethroids and surrogate is prepared by diluting 2.5 mL of the pyrethroid stock solution (40 ng/µL) plus 1 mL of the surrogate stock solution (100 ng/µL) followed by 2 mL of internal standard stock solution (10 ng/µL) into EtOAc in a 10-mL volumetric flask.

7.5 *Internal standard solution*—Solution containing 2 ng/µL of ISTD is prepared by diluting 10 mL of internal standard stock (10 ng/µL) into EtOAc in a 50-mL volumetric flask.

7.6 *Calibration solutions*—Prepare a series of (no fewer than 5) calibration solutions in EtOAc that contain all of the pyrethroids and the surrogate at concentrations ranging from 0.0025 ng/µL to 2.5 µg/kg, with the internal standard maintained at a constant concentration of 2 ng/µL (GC/MS calibration range is 0.025 to 2.5 ng/µL and GC/MS/MS calibration range is 0.0025 to 0.25 ng/µL). The calibration solutions are made by adding the appropriate amount of pyrethroid standard solution (10 ng/µL) and ISTD solution (10 ng/µL) in 5-mL volumetric flasks and bringing to volume with EtOAc.

7.7 *Matrix-spike solution*—Solution containing 2 ng/µL of pyrethroids is prepared by diluting 0.5 mL of pyrethroid stock solution (40 ng/µL) into EtOAc in a 10 mL volumetric flask.

7.8 *Surrogate spike solution*—Solution containing 2 ng/µL of surrogate is prepared by diluting 0.4 mL of surrogate stock solution (50 ng/µL) into EtOAc in a 10 mL volumetric flask.

8. Sample Preparation Procedure

The extraction of pyrethroids from water samples and sediment samples, and subsequent cleanup, are outlined below:

8.1 *Water sample preparation*

8.1.1 *Sample collection and storage*—Field-sampling procedures need to follow those typically used to collect samples for trace organic compound analyses (U.S. Geological Survey 2006; Ward and Harr, 1990) and special procedures unique to pyrethroids (Hladik and others, 2009). Samples are preferably filtered in the field. Samples are stored by refrigerating at 4 °C. Samples should be extracted within 48 hours of collection.

8.1.2 *Sample filtration*—If not filtered in the field, filter the 1-L water samples through a GF/F filter. Once the water has passed through the filter, measure the exact volume of water with a graduated cylinder and pour the water back into the original sample bottle.

8.1.3 *SPE extraction*—Stack the Oasis HLB SPE cartridges onto a vacuum manifold. Clean the cartridges with two column volumes of EtOAc. Condition the cartridges with two column volumes of MeOH and one column volume of deionized water. The filtered water sample is pumped through the SPE cartridge at a flow rate of 10 mL/min. The SPE cartridge is then dried under carbon dioxide for approximately one hour or until SPE sorbent is dry. The analytes are eluted into a concentrator tube using 12 mL of EtOAc.

8.1.4 *Bottle rinse*—Any remaining water in the sample bottle is absorbed with about 1 g of sodium sulfate (which is left in the bottle). The bottle is rinsed three times with 4 mL of DCM (collected in a concentrator tube) to remove any pyrethroids associating with the container walls.

8.1.5 *Sample concentration*—The bottle rinsate is reduced under a gentle stream of nitrogen using the N-evap to about 0.5 mL. The bottle rinsate is added to the SPE extract and then is reduced using the N-evap to approximately 0.2 mL. Add 40 µL of the 2-ng/µL ISTD solution and transfer to GC/MS vial (2-mL vials with 250-µL glass insert). The sample extracts are stored in a freezer at –20 °C until analysis (up to 30 days).

8.2 *Sediment sample preparation*

8.2.1 *Sample collection and storage*—Collect bed-sediment, aqueous suspended-sediment, and soil samples using methods that accurately represent the organic concentrations at a given location. Field-sampling procedures need to follow those typically used to collect samples for trace organic compound analyses (Ward and Harr, 1990; Radtke, 2005) and special procedures unique to pyrethroids (Hladik and others, 2009). Samples are immediately chilled, and at the laboratory, they are stored by freezing to –20 °C. A six-month holding time limit has been established (prior to sample extraction) from the date of sample collection. All samples are thawed before analysis.

8.2.2 *Microwave extraction*—Turn on the MAE and allow to warm up for 20–30 min; make sure to put the vent hose in the hood. Rinse all MAE vessels and caps with DCM and acetone before use. Start with wet (not dried) sediment; if frozen, thaw overnight in refrigerator. Prior to extraction, the percentage moisture of the sediment is calculated. Weigh approximately 5.0 g dry weight (calculate how much wet weight equivalent) of homogenized material into precleaned vessels labeled "MAE." Add 50 µL of 2 ng/µL surrogate solution. Adjust the percentage moisture of the sediment to no less than 50 percent by adding the appropriate amount of deionized water. If the sediment has over 50 percent moisture, do not add water to the sediment. Add 30 mL DCM: MeOH (90:10 ratio; premixed) to each sample vessel and insert vessel into sleeve. Cap the MAE vessels tightly and place into the MAE, and make sure the vessels are spaced evenly throughout the tray. Load the "sediment" method (for example, Sediment: 75% power; time: 20 min; TAP: 10 min; temperature: 120 °C). Once the MAE is done running the method, let cool for about 20 min (pressure needs to be less than 5 psi before removing MAE vessels). Set up glass funnels (with glass wool at bottom of funnel) with sodium sulfate (about 30 g). Vent the MAE vessels slowly to release remaining pressure. Open the extraction vessels and slowly decant the samples over sodium sulfate to remove the water and let the solvent flow into an appropriate collection vessel. Rinse the sodium sulfate two times with DCM (approximately 5 mL), collecting the DCM in the collection vessel corresponding to the sample. Once rinsed, discard the sodium sulfate. Repeat the microwave extraction on the sediment remaining in microwave vessels using 30 mL DCM:MeOH (90:10). Do not add more water to the sediment unless it looks dry. When completed and cooled, decant the extract through fresh sodium sulfate and into the same collection vessel (use the same procedure as used for the first extraction). Concentrate extracts to <0.5 mL using the TurboVap.

8.2.3 *SPE removal of matrix*—The first step is to remove matrix interferences using carbon and alumina SPE cartridges. Stack carbon SPE cartridges onto alumina SPE cartridges on a vacuum manifold. Clean cartridges with three column volumes of DCM. IMPORTANT: do not allow cartridges to go dry. After the cartridges are washed, place 12-mL glass graduated test tubes in the manifold rack. Add the sample (MAE extract) directly to top of carbon cartridge and rinse TurboVap tube with a small volume of DCM (<0.5 mL) to remove any remaining extract. Elute the analytes from the cartridges with 10 mL of DCM at ~1–2 drops/s. Reduce DCM fraction using the N-evap to 0.5 mL and exchange two times to EtOAc; final sample volume should be ~0.5 mL.

8.2.4 *HPLC/GPC removal of sulfur*—The second cleanup step, done to primarily remove sulfur, is accomplished with HPLC-GPC. Turn on pump and UV/Vis lamp (254-nm absorbance wavelength) and allow them to warm up for 30 min (flow rate = 1.0 mL/min). Make sure EtOAc reservoir is full. To determine the collection window (time interval), inject 200 µL of matrix-spike solution (2 ng/µL). Immediately following the injection, start the stopwatch. Once the ultraviolet (UV) absorbance starts to increase, note the time. When the absorbance drops back to approximately zero, note the time again; this will give you a collection window. To make sure all of the compounds have had sufficient time to exit the system, give the window a 30-s buffer on each side. Usually the collection window ranges from 7 to 15 min. Rinse the injector loop between samples with EtOAc. After determining the collection window, inject the entire sample onto the GPC and make sure to note the injection volume on the lab form. Immediately after the sample is injected, start the stopwatch. Place a 12-mL graduated test tube in the collection beaker and remove the waste hose at the start of the collection window. At the end of the collection window, re-attach the waste hose and allow solvent to pump through the GPC for another 30–35 min (sulfur should come out ~20 min after the end of your collection window). Reduce the resulting cleanedup sample to 0.2 mL, add 40 µL of ISTD, and transfer to GC/MS autosampler vials. The sample eluents are stored in a freezer at –20 °C until analysis.

9. Instrument Calibration and Analysis Procedures

Aliquots of the samples are injected and the compounds separated on a Varian CP-3800 GC and Saturn 2000 MS system with a DB-5ms analytical column (30 m × 0.25 mm × 0.25 µm).

9.1 *GC/MS performance evaluation*—Before a sample batch is run, a new injector insert and septa are installed on the GC, and approximately 10 cm are removed from the injector end of the analytical column to maintain column performance with sediment samples. For the MS, air and water leaks (mass to charge ratios, or *m*/*z* of 28 and 32, and 18, respectively) are checked prior to running. The MS calibration standard, perfluorotributylamine (PFTBA), is also used to optimize peak shape and calibrate the masses after instrument maintenance. The performance of the GC/MS is evaluated prior to each sample batch by injecting 1 µL of a pyrethroid calibration solution (0.5 ng/µL) and assessing retention times, peak areas, and product ion abundances and ratios (using the conditions described below).

9.2 *GC/MS injections for analysis*—The GC/MS and GC/MS/MS conditions for the analysis of pyrethroids are listed below.

9.2.1 *GC conditions*—Injections of 1 µL are made with the injector at 275 ºC in splitless mode with a 50 psi pressure pulse for 1 min. The flow of He through a GC column is set at 1.2 mL/min. The oven program is 80 ºC for 1 min, ramp at 10 ºC/min until 300 ºC, and hold for 10 min.

9.2.2 *MS conditions*—The transfer line from the GC to the MS is set at 280 ºC and the MS ion trap is set at 220 ºC. The MS is operated in electron ionization (EI) mode with an emission current of 45 µA with a multiplier offset of 300 volts (emission current was reduced to 15 µA and no offset for the internal standards). Data are collected in the selected ion storage (SIS) mode; details of the SIS windows are given in *table 2*.

9.2.3 *MS/MS conditions*—The instrument is operated in EI mode with an emission current of 50 µA and no multiplier offset. The isolation window is 3.0 *m*/*z*. Automated method development is used to determine the excitation amplitude for nonresonant ionization of the parent ion. Further MS/MS details are given in *table 3*.

9.3 *Instrument calibration*—The GC/MS in calibrated with each new sample batch. A number of calibration standards are run; a minimum of five and up to seven. The calibration range for GC/MS is 0.025 to 2.5 ng/µL and the calibration ranged for GC/MS/MS is 0.0025 to 0.25 ng/µL. These calibrations correspond to environmental sample concentrations of 0.5 to 500 ng/L for water and 0.2 to 100 µg/kg for sediment.

9.4 *Data acquisition and processing*—Varian Workstation software is used to calibrate and quantify the responses of the pyrethroids. Pyrethroids with multiple peaks (allethrin, cyfluthrin, cypermethrin, τ-fluvalinate, permethrin, resmethrin, and tetramethrin) are added together for quantitation. Calibration and quantitation are described in more detail in section 11.

10. Quality Assurance and Quality Control

The quality-assurance (QA) and quality-control (QC) program primarily consists of internal checks on precision and accuracy of analytical results. Laboratory quality-control data from continuous calibration verification (CCV), laboratory blanks and matrix-spiked samples, and internal and surrogate standards are used by the analyst to determine if corrective actions are needed or if sample concentrations are not accurately reported.

10.1 *Field sampling*—Accuracy of sample handling in the field is monitored when field blanks and field replicates are included for analysis by the laboratory. Each environmental sample or quality-control sample is handled separately for proper data determination by the analyst.

10.2 *Continuous calibration verification* (CCV)—The CCV solutions, which are standard solutions of pyrethroids prepared in a manner similar to the calibration standards, are used to monitor the method stability in comparison to the initial calibration curve. The CCV control limits are established at ± 25 percent of the expected concentration for each pyrethroid. If a CCV fails the QC criteria, the affected samples are reanalyzed.

10.3 *Internal standards*—Internal standards are added to correct quantitative differences in extract volume as well as to compensate for differences in extract volume injected. They are also used to monitor instrument conditions, such as extract injection errors, retention time shifts, or instrument abnormalities or malfunctions.

10.4 *Laboratory blank*—A laboratory blank is an aliquot of either deionized water (for water samples) or baked sodium sulfate (for sediment samples) used to monitor the entire sample preparation and analytical procedure for possible laboratory contamination. The laboratory blank is considered acceptable when a compound is either undetected, or is detected at or below one-third of the MDL. On the basis of data collected during the development of this method (10 water blanks and 22 sediment blanks), there are no interferences in the laboratory blanks. Laboratory blanks are analyzed at a minimum of 1 per every 20 samples. If a compound is detected in the laboratory blank above the MDL, then no further samples are run until the source of the contamination is identified and eliminated.

Table 2. Retention times, number of GC peaks, selected ion storage levels, and quantitation ions for pyrethroids and internal standards analyzed by GC/MS.

[The selected ion storage (SIS) levels are the ion ranges the instrument stores; compounds with multiple quantitation ions are added together to increase sensitivity. GC/MS is ion trap. GC, gas chromatography; GC/MS, gas chromatography with mass spectrometry; min, minute; *m*/*z*, mass-to-charge ratio]

Compound	Retention time (min)	Number of GC peaks	SIS storage levels (*m*/*z*)	Quantitation ions (*m*/*z*)	Confirmation ions (*m*/*z*)
d_{10}-Acenaphthene	10.3	1	90-450	162	—
Tefluthrin	14.0	1	175-179, 195-201	177	141, 197
d_{10}-Phenanthrene	14.9	1	90-450	188	—
Allethrin	16.6	2	89-95, 121-125	123	91,136
d_{10}-Pyrene	17.2	1	90-450	212	—
Resmethrin	19.5	2	95-146, 163-179	143+171	123
Bifenthrin	20.0	1	93-100, 119-143, 158–186, 195-201, 262-269	181	165, 166
Tetramethrin	20.1	2		164	123
Fenpropathrin	20.2	1		181+265	125
Sumithrin (phenothrin)	20.5	1		123+183	237
λ–Cyhalothrin	20.9	1		181	197, 225
Permethrin	21.9	2	89-95, 149-170, 178-201, 224-229	183	127, 163
Cyfluthrin	22.4	4		127+163+199	—
Cypermethrin	22.7	4		127+163+181	—
τ–Fluvalinate	23.7	2	123-129, 149-156, 165-184, 223-228, 248-257	250	167, 181
Esfenvalerate	23.8	1		225	181, 252
Deltamethrin	24.5	1		253	172, 181

10.5 *Laboratory matrix spike*—The laboratory matrix spike is an aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The laboratory matrix spike is analyzed exactly like a regular sample and is used to determine whether the sample matrix contributes bias to the analytical results and, therefore, to what degree the method is successful in recovering the target analytes. The background concentrations of the analytes in the sample matrix, if any are present, must be determined in a separate aliquot so that the values in the laboratory matrix spike can be corrected for background concentrations and the percentage recovery calculated. Laboratory matrix spikes are analyzed at a minimum of 1 per every 20 samples, or more frequently if a batch includes new or usual sample matrixes. If a matrix spike is below 70 percent, then the sample set is evaluated for potential issues; if these issues cannot be rectified, the sample set is thrown out.

10.6 *Laboratory matrix-spike duplicate*—The laboratory matrix-spike duplicate is prepared and analyzed in the same manner as the laboratory matrix spike and is compared with the laboratory matrix spike to determine method variability. Laboratory matrix spikes are analyzed at a minimum of 1 per every 30 samples if the study calls for laboratory matrix-spike duplicates. The matrix-spike duplicate must be have a relative percent deviation less than 25 percent to be considered acceptable.

Table 3. Analysis and quantitation parameters for pyrethroids analyzed by GC/MS/MS

[GC/MS/MS is ion trap. GC/MS/MS, gas chromatography with tandem mass spectrometry; *m*/*z*, mass-to-charge ratio]

Compound	Parent ion (*m*/*z*)	Excitation storage level (*m*/*z*)	Nonresonant excitation amplitude (volts)	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 (phenothrin)	183	80.5	75	153+168+181
Tefluthrin	177	77.9	80	117+127
Tetramethrin	164	72.1	61	77+91+107

10.7 *Laboratory replicate*—The laboratory replicate is a sample that is split into fractions for multiple analyses. Laboratorymatrix spikes are analyzed at a minimum of 1 per every 20 samples.

10.8 *Surrogate standards*—Surrogate standards are compounds similar in physical and chemical properties to the method compounds, but which are not expected to be present in the environment. They are added to each environmental and quality assurance quality control (QA/QC) sample and used to monitor matrix effects and overall method performance. Their recoveries are not used to correct compound concentrations in environmental samples. If surrogate recoveries are less than 70 percent, the sample is either thrown out (if there is no more sample material) or reextracted and analyzed (if more sample material is available.

10.9 *Solvent blank*—An injection of solvent (in this case EtOAc) is made onto the GC/MS to determine if there is carryover of target analytes between sample injections. If analytes are detected in the solvent blank, the source of the carryover is determined and the sample set is repeated.

10.10 *Instrumental analysis quality control*—An example of a typical analytical sequence used for this method is listed in *table 4*. Sample extracts (including field blanks, replicates, matrix spikes, and laboratory spikes) are analyzed in an instrument sequence to provide additional information to facilitate corrective actions that might be required if performance criteria are not met.

11. Calculation of Results

Before quantitative results are reported, each compound first needs to meet qualitative criteria:

11.1 *Qualitative identification*—Identification and quantitation of compounds are performed on the raw data files using the Varian Workstation data analysis package. A compound is not considered to be identified correctly unless the correct quantitation ion(s) of the peak are detected, the relative ratios of the confirmation ions are within ±25 percent of the average ratio obtained from the calibration samples, and the peak's relative retention time is within 5 percent of the expected retention time. All sample extracts are first analyzed in GC/MS mode; if all pyrethroids that are detected in an extract are at concentrations above the MDL, then no further analyses are performed for that extract. If one or more pyrethroids are detected at concentrations below the GC/MS MDL, or if a quantitation ion is present but without the proper ratios of confirmation ions, that extract is reanalyzed in GC/MS/MS mode and evaluated.

Table 4. Example analytical sequence for use in determining pyrethroids in sediments.

[Samples listed in column three include blanks (field and laboratory), replicates (field and laboratory), matrix spikes, and matrix-spike duplicates. CCV, continuing calibration verification; EtOAc, ethyl acetate]

Sample number	Vial number	Sample type
1	1	Solvent blank (EtOAc)
2	2	Calibration standard 1
3	3	Calibration standard 2
4	4	Calibration standard 3
5	5	Calibration standard 4
6	6	Calibration standard 5
7	7	Calibration standard 6
8	8	Calibration standard 7
9	1	Solvent blank (EtOAc)
10	9	Sample 1
11	10	Sample 2
12	11	Sample 3
13	12	Sample 4
14	13	Sample 5
15	14	Sample 6
16	6	CCV
17	1	Solvent blank (EtOAc)
18	15	Sample 7
19	16	Sample 8
20	17	Sample 9
21	18	Sample 10
22	19	Sample 11
23	20	Sample 12
24	6	CCV
25	1	Solvent blank (EtOAc)
26	21	Sample 13
27	22	Sample 14
28	23	Sample 15
29	24	Sample 16
30	25	Sample 17
31	26	Sample 18
32	6	CCV
33	1	Solvent blank (EtOAc)

In GC/MS/MS mode, there is the potential for the background to cause incomplete disassociation of the parent ion, which can interfere with proper quantitation. The excitation amplitude for MS/MS was set to a level that was optimal for the responses of the quantitation ions(s), typically resulting in the parents ions occurring at about 5 percent relative abundance. If the relative ratios of the confirmation ions are not within ±25 percent of the average ratio obtained from the calibration samples, then the analyst assumes that there was incomplete dissociation of the parent ion. For this reason, the sample extracts are run first using only GC/MS, and then pyrethroids below the MDLs are rerun using GC/MS/MS.

Estimated GC/MS concentrations are compared to those quantitated using GC/MS/MS to eliminate the concern of matrix interferences. If the GC/MS/MS concentrations differ by more than 25 percent of the GC/MS concentration, and there is complete dissociation of the parent ion in the GC/MS/MS spectra, it is assumed that there is matrix interference, and the GC/MS concentration is used for quantification. If this concentration is below the GC/MS MDL, the concentration is listed as "estimated."

11.2 *Quantitation*—Only after the compound has passed qualitative criteria is the concentration calculated according to a calibration curve used to establish the best fit between the calibration points. Five- to six-point calibration curves are constructed using linear regression from the calibration standards (which standards are used depends on sample concentrations and instrument performance). The correlation coefficient for each standard curve has to be greater than or equal to 0.99 to be accepted. The response factor for each compound is calculated from the calibration curve.

11.2.1 *Response-factor calculation*

Calculate the response factor (RF) for each selected compound as follows:

$$RF = \frac{C_c \times A_i}{C_i \times A_c} \quad (1)$$

where

C_c = concentration of the selected compound in nanograms per microliter;

A_i = area of peak of the quantitation ion for the internal standard;

C_i = concentration of the internal standard in nanograms per microliter; and

A_c = area of peak of the quantitation ion for the selected compound.

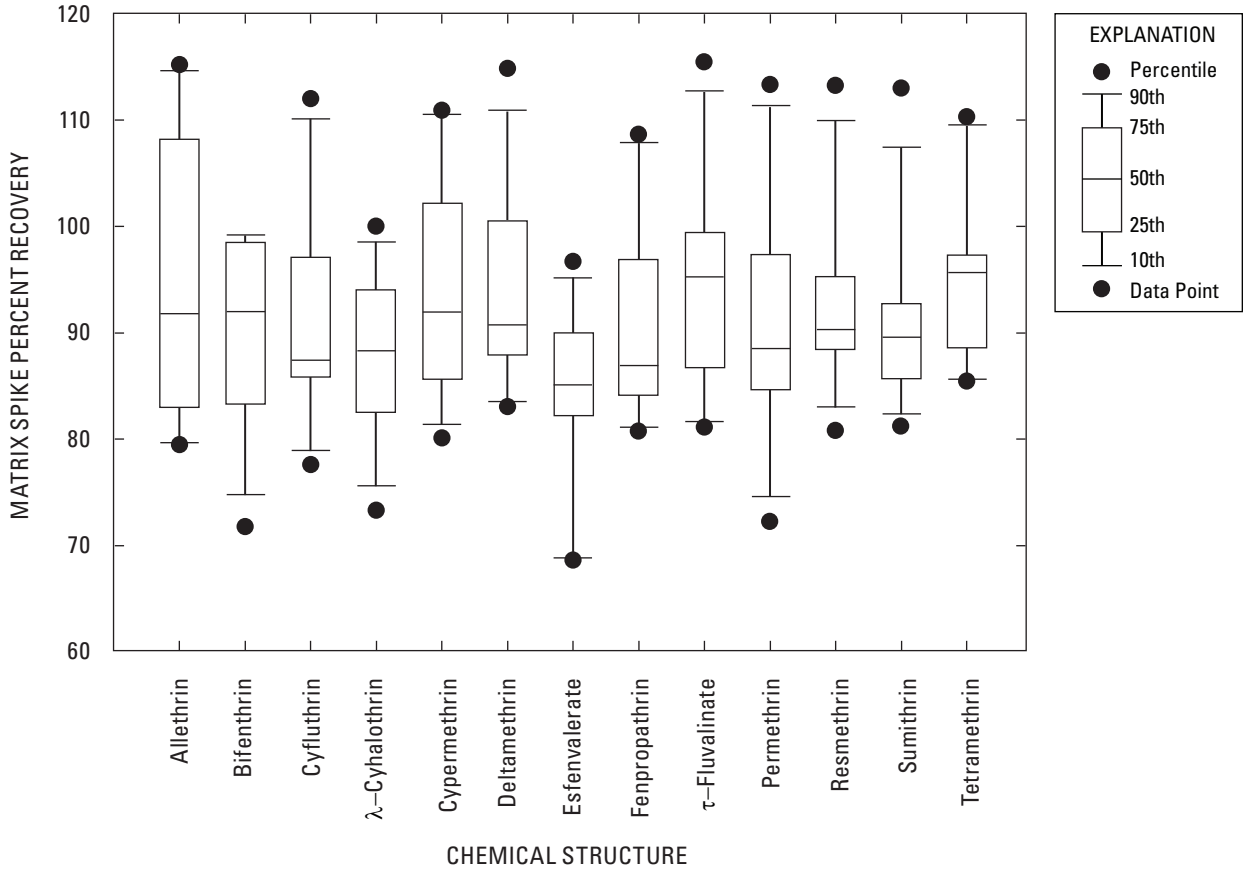

Figure 2. Results of the analysis of 12 matrix spikes of environmental water samples for pyrethroid insecticides.

11.3 *Calculations*—If a selected compound has passed the qualitative identification criteria, and the area under the peak(s) for the quantitation ion(s) for that compound has been properly integrated, the concentration in the sample is calculated as follows:

11.3.1 *Water-sample calculations*

Calculate sample-extract concentrations, *E*, for each compound:

$$E = (A_c / A_i)\ (RF)\ C_i \text{ , in nanograms per microliter} \quad (2)$$

where

E = concentration of the selected compound in the sample extract, in nanograms per microliter;
A_c = area of peak of the quantitation ion for the selected compound;
A_i = area of peak of the quantitation ion for the internal standard;
RF = response factor calculated in equation 1; and
C_i = concentration of the internal standard in nanograms per microliter.

Calculate sample concentrations, C, in nanograms per liter, for each compound:

$$C = (E \times 200\ \mu L)/V_s\ , \quad (3)$$

where

E = concentration of the selected compound in the sample extract, in nanograms per microliter; and
V_s = volume of water sample, in liters.

11.3.2 *Sediment-sample calculations*

Calculate the dry weight of sediment extracted, in grams (W_s):

$$W_s = W_w\ [(100 - \%\ \text{moisture})/100] \quad (4)$$

where

W_s = dry weight of sediment, in grams;
W_w = wet weight of sediment, in grams.

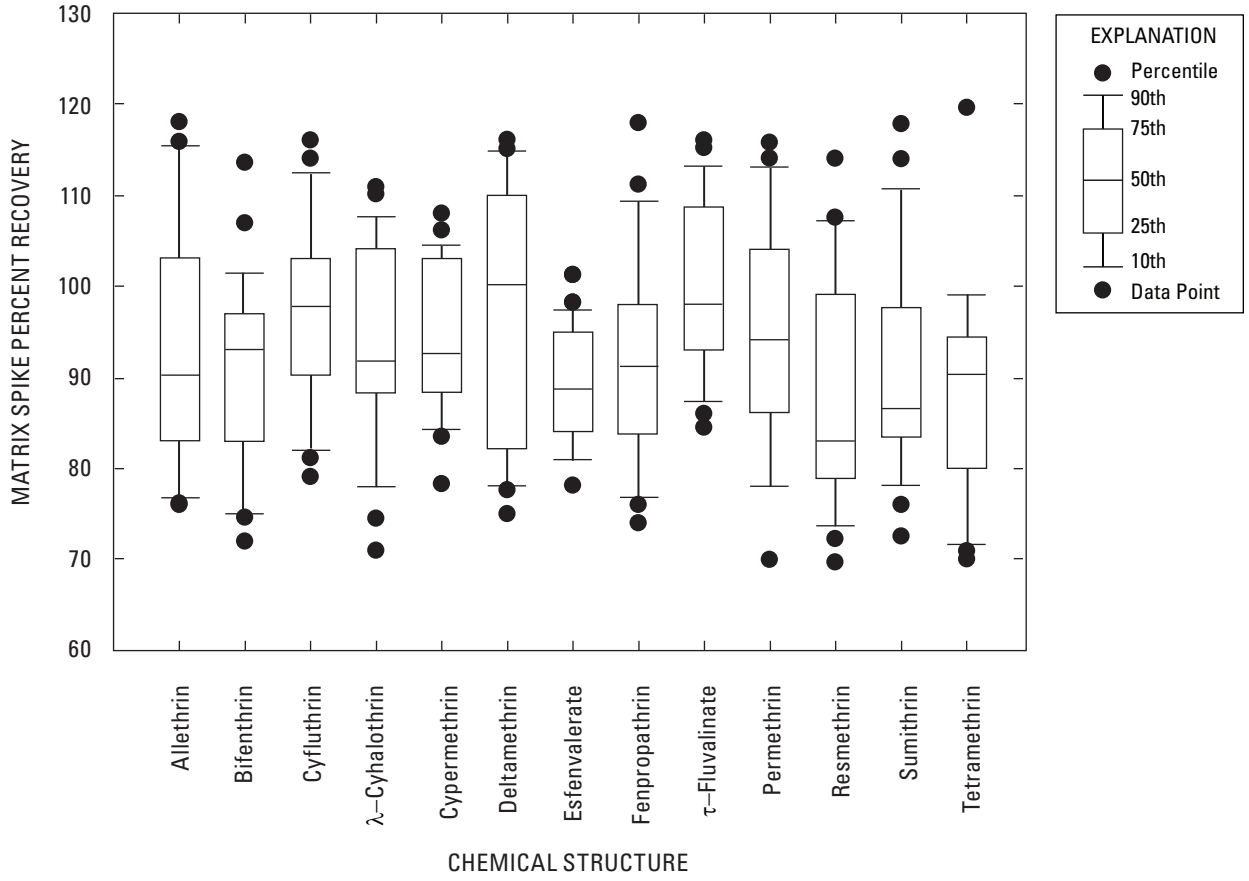

Figure 3. Results of the analysis of 27 matrix spikes of environmental bed sediment, suspended sediment, and soils for pyrethroid insecticides.

Calculate sample-extract concentrations, *E*, for each compound, in nanograms per microliter, using equation 2 in 11.3.1.

Calculate sample concentrations, C, in nanograms per gram (which is equal to micrograms per kilogram), for each compound:

$$C = (E \times 200\ \mu\text{L})/W_s, \quad (5)$$

where

E = concentration of the selected compound in the sample extract, in nanograms per microliter;

W_s = dry weight of sediment, in grams.

12. Reporting of Data Results

Pyrethroids are reported in concentrations from 0.5 ng/L to 500 ng/L for water and 0.2 to 100 µg/kg for sediment. If the concentration is greater than 500 ng/L or 100 µg/kg, a portion of the original sample extract is diluted appropriately with EtOAc, prepared with internal standard, and reanalyzed.

13. Method Performance

Initial method performance was evaluated for recovery using water collected from the American River that was spiked to 10 ng/L with pyrethroids (water), or sediment collected from a Northern California agricultural drain that was spiked to 10 µg/kg (sediment, dry weight). Neither of these sample matrixes had detectable levels of pyrethroids prior to spiking. MDLs were determined using seven samples spiked at either 10 ng/L or 10 µg/kg. Additional method performance was assessed through matrix spikes at 40 ng/L (water) and 40 µg/kg (sediment, dry weight).

13.1 *Method recovery*—Pyrethroid recoveries were determined by comparing seven spiked samples with one another. Pyrethroids were spiked into a several water and sediment matrices at 10 and 100 ng per 1 L of water or 5 g (dry weight) of sediment. *Table 5* shows the mean recoveries (of a theoretical 100 percent) for three water matrixes, two sediment matrixes, and one soil matrix. The water matrices were American River water, a surface drain, and an agricultural drain. The American River water was used in place of reagent water and this better represents clean real-world conditions. The American River is snowmelt and drainage from the Sierra Nevada, the water is detained by a series of dams upstream of the collection point, which makes this matrix water consistent in composition; the river has low suspended sediments and low dissolved organic carbon and has not had any pesticide detections in blank samples that have been run over several years in the laboratory. Method recoveries varied from 83 to 107 percent with relative standard deviations (RSDs) of 5 to 9 percent for American River water; the other two water matrices had recoveries of 70 to 108 percent (RSDs of 4 to 20 percent). The bed sediments had recoveries ranging from 82 to 101 percent with relative standard deviations of 3 to 9 percent for sediment; the other two matrices had recoveries of 82 to 102 (RSDs of 3 to 11 percent).

13.2 *Method detection limit (MDL)*—The MDL is defined as the minimum concentration of a substance that can be measured and reported with 99 percent confidence that the compound concentration is greater than zero (U.S. Environmental Protection Agency, 1997). Initial MDLs were determined according to the procedure outlined by the U.S. Environmental Protection Agency in 40 CFR 136, Appendix B, assuming a 1-L (water) or 5-g (sediment) sample size. For MDL determination, American River water and a bed sediment were used as the matrix.

The MDL was calculated according to the equation

$$\mathrm{MDL} = S \times t_{(n-1,1-\alpha=0.99)} \quad (6)$$

where

S = standard deviation of replicate analyses, in nanogram per liter or microgram per gram, at the lowest spike concentration;

n = number of replicate analyses; and

$t_{(n-1,1-\alpha=0.99)}$ = Student's t value for the 99 percent confidence level with n−1 degrees of freedom.

According to the USEPA procedure, at least seven replicate samples are fortified with compounds at concentrations two to five times the estimated MDL. This concentration range was used to calculate initial MDLs for the pyrethroids. All samples are first run via GC/MS for verification of pyrethroids and quantification. If the pyrethroid concentrations are lower than the GC/MS MDLs, the sample extracts are run on the GC/MS/MS to decrease background noise. In samples with high background matrix (especially in sediment samples), the pyrethroid may not undergo complete dissociation by GC/MS/MS, confounding the calculation of analyte concentrations. Samples that are run in both modes will have their concentrations compared as described in section 11.1; if the sample run by GC/MS/MS has spectra where the parent ion has not undergone complete dissociation, there may be background interferences, and the GC/MS data (and associated MDLs) will be reported. The MDLs for the tandem mass spectrometry analyzed samples are lower (0.5 to 1.0 ng/L for water and 0.2 to 0.5 µg/kg sediment) than those for the single mass spectrometry samples (2.0 to 6.0 ng/L for water and 1.0 to 2.6 µg/kg for sediment) (*table 6*).

The MDLs were also compared with the theoretical limits of detection (LOD). The LOD was calculated as the concentration of analyte in the spiked sample that produced a signal greater than three times the background signal. For each method using GC/MS, the LODs were 2 to 5 ng/L for water and 1 to 2 µg/kg for sediment. The MDLs are higher than the LODs because MDLs take into account sample recovery and variability rather than the theoretical lowest concentration that can be measured.

13.3 *Matrix performance*—To evaluate potential matrix effects on analyte recovery, matrix-spiked samples were collected from varying locations and analyzed over a period of four years (2005–2008). Water samples were taken from stream and creeks in agricultural and urban areas. Sediment samples consisted of bed sediments, suspended sediments, and soils. Percentage organic carbon content of the sediment samples ranged from 0.2 to 8 percent. For each matrix spike, a paired unspiked sample was extracted to determine baseline pyrethroid concentrations.

Figure 2 shows box plots of the environmental water matrix spikes (total of 12 samples). Percentage recoveries (minus any baseline concentrations) ranged from 84 to 96 percent, with standard deviations of 7 to 13 percent. *Figure 3* shows box plots of the environmental sediment matrix spikes (total of 27 samples). Percentage recoveries (minus any baseline pyrethroid concentrations) ranged from 88 to 100 percent with standard deviations of 6 to 14 percent. Tefluthrin has recently been added to the method and is not shown because of insufficient data. The method worked well over time and for sediments with varying organic carbon content.

Table 5. Summary of mean-percent recoveries of theoretical spiked concentrations and relative standard deviations for pyrethroids in three water and two sediment and soil matrices.

[Suspended sediments were spiked at only one concentration as it is hard to get a large volume of suspended sediments for spiking experiments. n = 7 for all matrices. ng, nanogram; n, number of samples; RSD, relative standard deviation; %, percent]

Compound	Spike amount (ng)	American River (reagent)		Surface water		Agricultural drain		Bed sediment		Suspended sediment		Soil	
		%Recovery	%RSD	%Recovery	%RSD	%Recovery	%RSD	%Recovery	%RSD	%Recovery	%RSD	%Recovery	%RSD
Allethrin	10	107	7	107	9	84	15	82	7			94	8
	100	95	3	90	11	96	13	93	1	94	6	97	7
Bifenthrin	10	94	6	93	8	70	5	97	7			97	9
	100	94	2	82	8	93	7	96	8	99	5	95	8
Cyfluthrin	10	89	9	98	8	97	19	82	6			98	8
	100	95	2	87	10	93	10	87	9	94	8	94	9
λ-Cyhalothrin	10	85	9	85	7	73	10	89	9			95	9
	100	93	2	84	9	90	7	94	6	93	10	95	9
Cypermethrin	10	85	8	85	10	99	16	87	8			94	9
	100	92	2	94	9	94	11	92	8	97	11	96	8
Deltamethrin	10	96	9	97	6	87	12	82	8			82	8
	100	91	3	92	9	94	10	93	9	101	4	86	7
Esfenvalerate	10	89	8	91	7	92	8	83	8			85	6
	100	93	3	88	4	83	10	89	7	90	5	89	6
Fenpropathrin	10	88	9	85	8	107	13	90	6			94	6
	100	95	3	91	9	90	10	87	9	98	6	95	9
τ-Fluvalinate	10	83	9	86	10	72	11	99	9			94	9
	100	93	5	100	7	93	8	97	9	102	5	91	6
Permethrin	10	98	8	99	11	86	14	93	3			93	7
	100	94	3	81	8	94	11	90	5	98	9	97	9
Resmethrin	10	92	8	92	10	74	20	89	6			93	10
	100	91	6	98	11	91	6	86	7	93	5	89	6
Sumithrin (phenothrin)	10	99	8	98	8	108	12	101	3			91	6
	100	95	5	91	3	91	10	93	6	94	6	95	6
Tefluthrin	10	96	7	97	5	91	6	91	4			97	7
	100	94	3	94	7	93	8	94	5	96	4	93	6
Tetramethrin	10	95	5	94	4	87	4	83	4			82	6
	100	98	1	101	11	94	4	84	6	94	3	86	4

Table 6. Percent recovery, relative standard deviation, and method detection limits for pyrethroids in water and sediment.

[Sediment is based on dry weight. GC/MS, gas chromatography with mass spectrometry; GC/MS/MS, gas chromatography with tandem mass spectrometry; MDL, method detection limit; ng/L, nanogram per liter; RSD, relative standard deviation; µg/kg, microgram per kilogram]

Compound	Water				Sediment			
	Mean recovery (percent)	Relative standard deviation (percent)	MDL GC/MS (ng/L)	MDL GC/MS/MS (ng/L)	Mean recovery (percent)	Relative standard deviation (percent)	MDL GC/MS (µg/kg)	MDL GC/MS/MS (µg/kg)
Allethrin	107	7	6.0	1.0	82	7	1.5	0.2
Bifenthrin	94	6	4.7	0.7	97	7	2.2	0.2
Cyfluthrin	89	9	5.2	1.0	82	6	2.0	0.5
λ-Cyhalothrin	85	9	2.0	0.5	89	9	2.4	0.2
Cypermethrin	85	8	5.6	1.0	87	8	2.6	0.4
Deltamethrin	96	9	3.5	0.6	82	8	2.5	0.2
Esfenvalerate	89	8	3.9	0.5	83	8	2.1	0.2
Fenpropathrin	88	9	4.1	0.6	90	6	2.1	0.2
τ-Fluvalinate	83	9	5.3	0.7	99	9	2.6	0.2
Permethrin	98	8	3.4	0.6	93	3	1.0	0.2
Resmethrin	92	8	5.7	1.0	89	6	1.9	0.5
Sumithrin (phenothrin)	99	8	5.1	1.0	101	3	1.3	0.3
Tefluthrin	96	7	4.8	0.6	91	4	1.1	0.2
Tetramethrin	95	5	2.9	0.5	83	4	1.4	0.2

13.4 *Method variability*—Method variability was evaluated by the use of laboratory replicates and matrix-spike/matrix-spike duplicate pairs. Variability was determined as relative percentage difference, RPD, calculated as:

$$\mathrm{RPD} = \frac{|C_1 - C_2|}{(C_1 + C_2)} \times 100 \text{ percent} \qquad (7)$$

where

C_1 is the concentration in one sample of the pair, and

C_2 s the concentration in the other sample of the pair.

Variabilities between replicate analyses over the 2005–2008 period ranged from 0 to 25 percent, with a median of 10 to 15 percent for the 14 pyrethroids.

Summary

This method provides details for the analysis of 14 pyrethroid insecticides in environmental water and sediment samples. The pyrethroids are isolated from the filtered water samples via solid-phase extraction; a bottle rinse must be included to recover any pyrethroids that associated with the sample container during the sample pumping. Pyrethroids were isolated from sediment samples by microwave-assisted extraction with an organic solvent, the co-extracted matrix is removed via carbon/alumina solid-phase extraction, and sulfur is removed via high-performance liquid chromatography and gel-permeation chromatography. Quantitation is achieved with gas chromatography and mass spectrometry (GC/MS) or gas chromatography and tandem mass spectrometry (GC/MS/MS).

The analytical method showed good precision, with greater than 80 percent recovery and standard deviations less than 10 percent for a single matrix (water or sediment). Among varying environmental water and sediments matrices, recoveries were greater than 84 percent for all pyrethroids in both water and sediment. Method detection limits (MDLs) for individual compounds in water or sediment ranged from 0.5 to 1.0 ng/L or 0.2 to 0.5 µg/kg, respectively for GC/MS/MS; and from 2.0 to 6.0 ng/L or 1.0 to 2.6 µg/kg, respectively for GC/MS.

References Cited

Bonwick, G.A., Sun, C., Abdullatif, P., Baugh, P.J., Smith, C.J., Armitage, R., Davies, D.H, 1995, Determination of permethrin and cyfluthrin in water and sediment by gas chromatography-mass spectrometry operated in the negative chemical-ionization mode: Journal of Chromatography A, v. 707, p. 293–302.

California Department of Pesticide Regulation, 2005, Pesticide use data for 2005 [digital data]: Sacramento, California, Department of Pesticide Regulation.

Esteve-Turrillas, F.A., Aman, C.S., Pastor, A., and de la Guardia, M., 2004, Microwave-assisted extraction of pyrethroid insecticides from soil: Analytical Chimica Acta, v. 522, p. 73–78.

Fernández-Gutiérrez, A., Martínez-Vidal, J.L., Arrebola-Liébanas, F.J., Gonzalez-Casado, A., and Vílchez, J.L, 1998, Determination of endosulfan and some pyrethroids in waters by micro liquid-liquid extraction and GC-MS: Fresenius Journal of Analytical Chemistry, v. 360, p. 568–572.

Hengel, M.J., Mourer, C.R., and Shibamoto, T, 1997, New method for analysis of pyrethroid insecticides: esfenvalerate, *cis*-permethrin, and *trans*-permethrin, in surface waters using solid-phase extraction and gas chromatography: Bulletin of Environmental Contamination and Toxicology, v. 59, p. 171–178.

Hill, I.R., 1989, Aquatic organisms and pyrethroids: Pesticide Science, v. 27, p. 429–465.

Hladik, M.L., Orlando, J.L., and Kuivila, K.M, 2009, Collection of pyrethroids in water and sediment matrices: Development and validation of a standard operating procedure: U.S. Geological Survey Scientific Investigations Report 2009-5012, 22 p. Available at *http://pubs.usgs.gov/sir/2009/5012/*

Hladik, M.L., Smalling, K.L., and Kuivila, K.M., 2008, A multi-residue method for the analysis of pesticides and pesticide degradates in water using HLB solid-phase extraction and gas chromatography-ion trap mass spectrometry: Bulletin of Environmental Contamination and Toxicology, v. 80, p. 139–144.

Houtman, C.J., Leonardsa, P.E.G., Kapiteijna, W., Bakkerc, J.F., Brouwera, A., Lamoreea, M.H., Leglera, J., and Klamerc, H.J.C, 2007, Sample preparation method for ER-CALUX bioassay screening (xeno-) estrogenic activity in sediment extracts: Science of the Total Environment, v. 386, p. 134–144.

Laskowski, D.A., 2002, Physical and chemical properties of pyrethroids: Reviews of Environmental Contamination and Toxicology, v. 174, p. 49–170.

LeBlanc, L.A., Orlando, J.L., and Kuivila, K.M., 2004, Pesticide concentrations in water and in suspended and bottom sediments in the New and Alamo Rivers, Salton Sea watershed, California: U.S. Geological Survey Data Series Report 2004-104, 15p.

Lee, S., Gan, J.Y., and Kabashima, J., 2002, Recovery of synthetic pyrethroids in water samples during storage and extraction: Journal of Agricultural and Food Chemistry, v. 50, p. 7194–7198.

Radtke, D.B., revised June 2005, The National Field Manual for the Collection of Water Quality Data, Bottom-material samples: U.S. Geological Survey Techniques of Water-Resources Investigations, book 9, chapter A8: Bottom sample materials. Available at *http://pubs.water.usgs.gov/twri9A/*

Sanchez-Brunete, C., Miguel, E., and Tadeo, J.L., 2002, Multi-residue analysis of fungicides in soil by sonication-assisted extraction in small columns and gas chromatography: Journal of Chromatography A, v. 976, p. 319–327.

Smalling, K.L., Orlando, J.L., and Kuivila, K.M., 2007, Occurrence of pesticides in water, sediment, and soil from Yolo Bypass, California: San Francisco Estuary and Watershed Science v. 5, Article 2.

Smalling, K.L., and Kuivila, K.M., 2008, Multi-residue method for the analysis of 85 current-use and legacy pesticides in bed and suspended sediments: Journal of Chromatography A, v. 1210, p. 8–18.

U.S. Environmental Protection Agency, 1997, Guidelines establishing test procedures for the analysis of pollutants (appendix B, part 136, Definition and procedures for the determination of the method detection limit): U.S. Code of Federal Regulations, Title 40, revised as of July 1, 1997, p. 265–267.

U.S. Geological Survey, 2006, The National Field Manual for the Collection of Water Quality Data, Collection of water samples (ver 2.0): U.S. Geological Survey Techniques of Water Resources Investigations, book 9, chap A4. Available at *http://pubs.water.usgs.gov/twri9A4/*

van der Hoff, G.R., Pelusio, F., Brinkman, U.A.T., Baumann, R.A., and van Zoonen, P., 1996, Automated solid-phase extraction coupled to gas chromatography with electron-capture detection: A combination of extraction and cleanup of pyrethroids in the analysis of surface water: Journal of Chromatography A, v. 719, p. 59–67.

Ward, J.R., and Harr, C.A. eds., 1990, Methods for collection and processing of surface-water and bed-material samples for physical and chemical analyses: U.S. Geological Survey Open-File Report 90-140, 71 p. Available at *http://pubs.er.usgs.gov/usgspubs/ofr/ofr90140*

You, J., and Lydy, M.J., 2004, Evaluation of desulfuration methods for pyrethroid, organophosphate, and organochlorine pesticides in sediment with high sulfur content: Archives of Environmental Contamination and Toxicology, v. 47, p. 148–153.

You, J., Weston, D.P., and Lydy, M.J., 2004, A sonication extraction method for the analysis of pyrethroid, organophosphate, and organochlorine pesticides from sediment by gas chromatography with electron-capture detection: Archives of Environmental Contamination and Toxicology, v. 47, p. 141–147.

Manuscript appproved on June 29, 2009
Prepared by the USGS Enterprise Publishing Network,
Publishing Service Center, Sacramento, California

For more information concerning the research in this report, contact the
California Water Science Center Director,
U.S. Geological Survey, 6000 J Street
Sacramento, California 95819-6129
http://ca.water.usgs.gov