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New Method for Analysis of Pyrethroid Insecticides: Esfenvalerate, *cis*-Permethrin, and *trans*-Permethrin, in Surface Waters Using Solid-Phase Extraction and Gas Chromatography

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Synthetic pyrethroids are used widely to control many common pests, including *Lepidoptera, Diptera,* and *Coleoptera* , on crops such as almonds, corn, garlic, and tomatoes (Meister, 1996). During the last 15 years, the use of synthetic pyrethroid insecticides, such as esfenvalerate and permethrin, has increased substantially, because of their potent insecticidal properties and low mammalian and avian toxicity (Ecobichon, 1996). In California, two commonly used pyrethroid insecticides are esfenvalerate or Asana®, *cis*-permethrin, and *trans*-permethrin. From 1990 through 1994, over 330,000 and 1.3 million lb of esfenvalerate and permethrin, respectively, were applied on various crops throughout California (Pesticide Use Report, 1990-1994).

Many aquatic organisms (fish, amphibians, macroinvertebrates, and microinvertebrates) are highly susceptible to pyrethroid intoxication, and applications typically occurring throughout the spring and summer months coincide with the spawning period of several fish species in California (Moyle, 1976). Esfenvalerate has exhibited detrimental effects to aquatic organisms, by reducing and/or eliminating test populations of crustaceans, chironomids, juvenile bluegills, and larval cyrinids at exposure levels of 1 ppb (Lozano, 1992). Permethrin, applied as a mixture of *cis*- and *trans*- isomers, has also been shown to adversely impact aquatic species, giving a LC_{50} (48 hr) of 5.4 ppb and 1.8 ppb for rainbow trout and bluegill sunfish, respectively (Kidd and James, 1995).

Since certain pyrethroids have been shown to enter the aquatic environment from agricultural runoff or drift from aerial or ground-based spraying (Tanner, 1996), applications may pose a serious threat to fish populations by direct exposure of young fish, which tend to be less tolerant to pesticides (Kumaraguru, 1981), and indirectly by the reduction of sensitive invertebrate populations serving as prey for young fish (Kreutzweiser, 1987). Therefore, it is important to develop a sensitive and selective method for determining these pesticides to monitor their possible contaminations in a surface water.

In the present study, a fast, selective, and sensitive method of esfenvalerate, *cis*-permethrin, and *trans*-permethrin analysis in surface waters using solid-phase extraction (SPE) and gas chromatography (GC) with electrolytic conductivity detection (ELCD) was developed.

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MATERIALS AND METHODS

Esfenvalerate [(S) α-cyano-3-phenoxybenzyl (S)-2-(4-chlorphenyl)-3-methylbutyrate] was purchased from DuPont (Wilmington, DE). *cis*- and *trans*-Permethrin [3-phenoxybenzyl (lRS)-*cis*- and -*trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate] were obtained from the EPA (Research Triangle Park, NC).

Surface waters (3 L) were collected from Putah Creek (South of UC Davis, Davis CA), Sacromento River (West of Sacramento Municiple airport, Sacromento, CA), Clear Lake (Konocti Marina, Kelseyville, CA), and Lake Tahoe (Near Elk Point, South Lake Tahoe, NV) in 4 L amber glass jugs. They were preserved on ice at the sampling site, through transit, and then stored in a 4°C refrigerator. Observed appearance and pH of the surface water samples were shown in Table 1.

Table 1. Observed appearance and pH of water samples.

Location collected	Collection date	Appearance	PH
Putah Creak	9/10/1996	Clouded	7.11
Sacramento River	9/ll/l996	Fine suspended solids	6.81
Clear Lake	9/29/1996	Clouded, suspended algae	6.55
Lake Tahoe	9/14/1996	Clear	5.82

Safety precautions were necessary to handle esfenvalerate because it is combustible at 66°C and can produce hydrogen cyanide. Proper protective clothing were worn when working with the compound.

A mixed stock solution of esfenvalerate, *cis*-permethrin, and *trans*-permethrin was prepared by dissolving 25.0 mg of each compound in acetone (J. T. Baker Inc., Phillipsburg, NJ) in a 25 mL volumetric flask and then the volume of a solution was adjusted to give a final concentration of 1 mg/mL concentration. Fortification standards of 100 µg/mL and 10 µg/mL solutions were prepared by transferring 1 mL of the stock solution into a 10 and 100 mL of acetone in volumetric flasks, respectively. The lowest fortification standard solution (1 µg/mL) was made by adding a 1 mL of the fortification solution (10 µg/mL) into 10 mL of acetone in a volumetric flask.

After water samples were equilibrated to room temperature, they were thoroughly mixed by shaking the 4 L jug for 5 min. Into a 125 mL erlenmeyer flask, 100 mL of water sample, fortification solution (if necessary), and 10 g of NaCl was added. The mixture was homogenized for 5 min with a gyrotory shaker (New Brunswick Scientific Company, New Brunswick, NJ). Samples were loaded into an attached 75 mL reservoir and passed through a C_{18} solid phase extraction (SPE) cartridges (Mega Bond-Elut®, 6 cc-l g, steel fritted, Varian, Harbor City, CA). The cartridges were conditioned by pulling through two column volumes each of methanol and deionized water with a vacuum manifold (Varian, Harbor City, CA) prior to use. In addition, the sample flask was rinsed with deionized water (10 mL) and the rinsate was loaded onto the cartridge. After all water was passed through, the cartridge was removed from the manifold, placed into a 15 mL graduated

centrifuge tube, and eluted with 6 mL ethyl acetate twice using a centrifuge (International Equipment Company, Needham Heights, MA).

Analysis of ethyl acetate extracts was conducted with a Hewlett-Packard Model 5890A GC (Hewlett-Packard, Avondale, PA) equipped with a O.I. Model 4420 electrolytic conductivity detector (O.I. Corporation, College Station, TX) and a 15 m x 0.53 mm i.d. (d_f=1.5 µm) Rtx-1® bonded phase fused silica megabore column (Restek Corporation, Bellefonte, PA). The injector and detector were operated at 250 and 280°C respectively. A Hewlett-Packard Model 6890 Series Autoinjector was used to inject 3 µL (set for fast injection) of samples in splitless mode. The oven temperature was programmed from 200°C to to 250°C at 5° C/min and held for 1 min. Helium was used as both carrier (20 mL/min) and make-up (10 mL/min) gas, and hydrogen was used as the detector gas (83 mL/min). Quantitation was performed by manually drawing baselines for each peak of interest and measuring the peak heights with Turbochrom® v4.1 software (Perkin Elmer Corporation, Norwalk, CT). Four point standard curves were used at the beginning and end of each set of samples with standards interspersed between replicate sample injections to ensure a linear response over the range of 100-800 pg/µL. The average of the standards was used to generate the standard curve for quantitation. Detection limits of qualitative and quantitative analysis were 30 pg and 300 pg for each pesticide, respectively.

Standard samples for the GC calibration curve were prepared by placing a 200 µL aliquot of the 100 µg/mL fortification solution into volumetric flasks containing a minimum amount of ethyl acetate and then the volume of the samples were brought up to 25, 50, 100, and 200 mL with ethyl acetate, resulting in 800, 400, 200, and 100 pg/µL standards, respectively.

In order to examine the stability of a sample during storage, the water samples from Putah Creek and Sacramento River (100 mL) were placed into 250 mL silanized amber bottles. The water samples were fortified with the pesticides at the 100 ppb level, and then stored in a 4°C refrigerator. Two replicates from each water sample were extracted and analyzed after 0, 1,2,4, and 8 days

RESULTS AND DISCUSSION

Advantages of combined SPE and ELCD method provides speed, simplicity, and sensitivity for determination of halogenated compounds, such as esfenvalerate and permethrin. Figure 1 shows a typical gas chromatogram of an ethyl acetate extract from a Lake Tahoe water fortified 1 ppb each of esfenvalerate, *cis*-, and *trans*-permethrin.

Table 2 shows results from method validation spikes of esfenvalerate and *cis*- and *trans*-petmethrin in deionized water. The permethrin recoveries were comparable to the recoveries conducted using a liquid-liquid extraction (Smith, 1983). The esfenvalerate recoveries were also consistent with those from agricultural runoff water tested using various elution patterns and C_{18} SPE packing sizes (Wells et al., 1994). Both methods described above use GC with electron capture detection (ECD) for quantification of the pesticides.

Although environmental water samples varied in pH and appearance (Table l), recoveries (Table 3) were not significantly different from those of deionized water (Table 2). This is somewhat surprising since suspended solid loading varied from

Table 2. Results of recovery tests on esfenvalerate and *cis*- and *trans*-permethrin from deionized water.

Concentration (µg/L)	Recovery (%)[a]		
	esfenvalerate	*cis*-permethrin	*trans*-permethrin
1	97 ± 8	85 ± 6	90 ± 8
10	95 ± 3	88 ± 2	88 ± 2
100	91 ± 6	91 ± 3	91 ± 4
1000	87 ± 4	91 ± 2	91 ± 2
Blank[b]	ND	ND	ND

[a] Average of six replicates. [b] None fortified deionized water. ND: not detected (less than 0.5 ppb).

Table 3. Results of recovery tests on esfenvalerate and *cis*- and *trans*-permethrin from surface water samples.

Water sample	Conc (µg/L)	Recovery (%)[a]		
		esfenvalerate	*cis*-permethrin	*trans*-permethrin
Putah Creak	1	92	90	92
	10	92	86	89
	100	97	95	96
	1000	88	87	88
	Control[b]	ND	ND	ND
Sacramento River	1	98	88	93
	10	88	80	93
	100	92	90	90
	1000	88	88	88
	Control	ND	ND	ND
Clear Lake	1	110	102	103
	10	90	80	84
	100	90	89	91
	1000	89	89	88
	Control	ND	ND	ND
Lake Tahoe	1	98	92	94
	10	92	84	86
	100	95	91	93
	1000	88	90	90
	Control	ND	ND	ND

a Average of two replicates. b None fortified surface water. ND: not detected (less than 0.5 ppb).

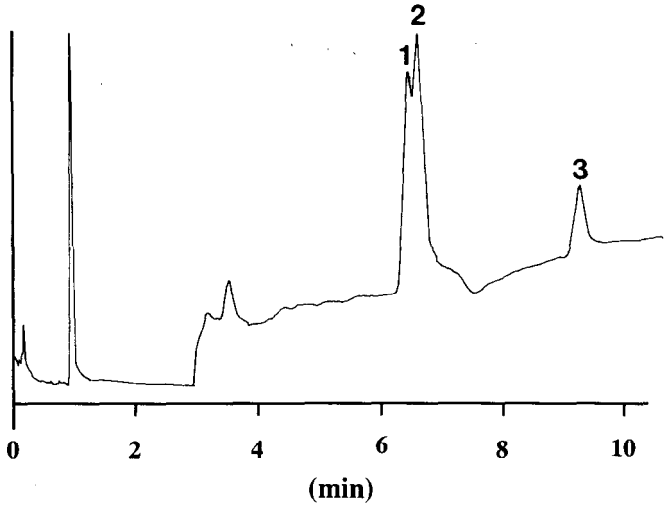

Figure 1. A typical gas chromatogram of an ethyl acetate extract from a Lake Tahoe water fortified 1 ppb each of cis-permethrin (**1** at 6.647 min) ,trans-permethrin (**2** at 6.799 min), and esfenvalerate (**3** at 9.539 min).

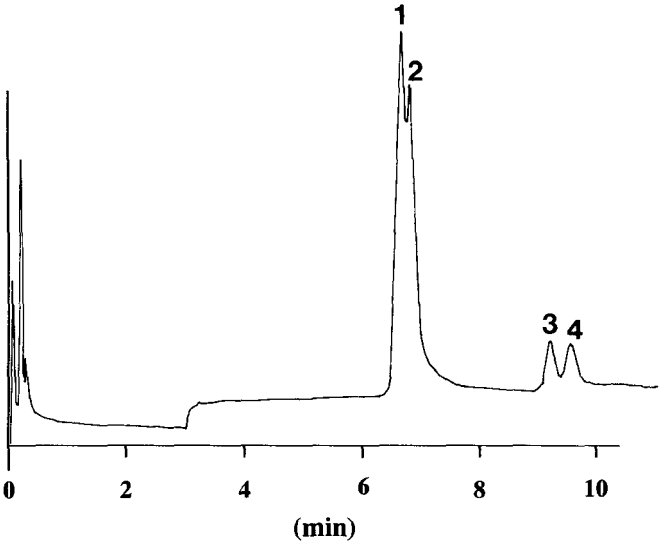

Figure 2. Gas chromatogram of an ethyl acetate extract obtained from a deionized water fortified with 10 ppb each of cis-permethrin (**1** at 6.633 min), *trans*-permethrin (**2** at 6.793 min), and esfenvalerate (**4** at 9.529 min). The peak at 9.183 min is fenvalerate.

highest to lowest in the order Clear Lake < Putah Creek < Sacramento River < Lake Tahoe. Also permethrin and esfenvalerate are highly lipophilic compounds which may partition from water to sediments in littoral enclosures (Heinis, 1992; Muir, 1985). Although it is possible that permethrin and esfenvalerate could sorb to suspended lipophilic particles and be carried through the C_{18} packing, no dramatic loss was shown in the recoveries (Table 3).

While ECD's sensitivity to halogenated compounds is single picograms over a linear range of two orders of magnitude, it may also respond to other electron accepting compounds in natural waters, such as aromatics, conjugated carbonyls, and organometallics (Willard et al., 1988). The detection of unwanted compounds may interfere determination of the analyte of interest. Alternatively, because ELCD provides extremely high selectivity for halogenated compounds, in addition to high sensitivity (10 pg) and large linear range (five orders of magitude), comparatively fewer chromatographic interferences are obtained (Willard et al., 1988). Additionally, SPE affords rapid sample through put, simultaneous processing of several samples, and reduced solvent waste compared to conventional liquid-liquid extraction methods.

A storage stability study was conducted to determine the acceptable storage time for natural water samples suspected of containing esfenvalerate, *cis*-permethrin, and *trans*-permethrin residues. A storage time of 2,4 and 8 days resulted significantly low recoveries (Table 4). During the storage stability study, ethyl acetate rinsates from the storage bottles revealed no permethrin or esfenvalerate residues, suggesting that the losses were not the result of pesticide adsorbing to the sample container. Eventhough approximately 60% of the permethrin was reportedly adsorbed on glass within 48 hr (Sharom and Solomon, 1981a). Thus, possible routes of loss may include physical adsorption on particulates suspended in the water samples, chemical degradation by oxidative free-radicals such as alkylperoxy and hydroxyl radicals present in natural waters, or microbial degradation (Sharom and Solomon, 1981b). It is advisable, therefore, that esfenvalerate and permethrin in natural water samples are analyzed within 24 hr.

Table 4. Results of stability test on esfenvalerate and *cis*- and *trans*-permethrin in surface waters during storage.

Water sample	Storage time (days)	Recovery (%)[a]		
		esfenvalerate	*cis*-permethrin	*trans*-permethrin
Putah Creak	0	95	91	91
	1	78	71	72
	2	37	37	38
	4	46	45	47
	8	40	44	45
Sacramento River	0	85	84	84
	1	71	68	71
	2	37	35	35
	4	48	44	45
	8	47	44	46

[a] Average of two replicates.

It was noted that a second peak eluted near the esfenvalerate peak in the screening studies (Wells, 1994). In the present study, a similar peak was found before esfenvalerate when the pH of the deionized water sample was adjusted above 8 with NaOH (Figure 2). This peak was later confirmed as fenvalerate, [(RS)-a-cyano-3-phenoxybenzyl (RS)-2-(4-chlorophenyl)-3-methylbutyrate] by GC-mass selective detection (Hewlett-Packard Model 5972A MSD coupled to a Hewlett-Packard Model 6890 GC). At no time during the analysis of natural waters did the fenvalerate peak occur and this is most likely due to the neutral to slightly acidic pH of the natural waters.

With increasing analytical demand for determination of pesticides in water in order to assess their possible hazard to aquatic organisms, the need for development of methods with selectivity, speed, and sensitivity have become paramount. The new method developed for esfenvalerate and *cis*- and *trans*-permethrin in the present study satisfies these criteria.

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