Small-Scale Method for the Determination of Permethrin Residue on Cattle Hair by Gas–Liquid Chromatography with Electron Capture Detection

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A simple small-scale method for the determination of permethrin on cattle hair by gas–liquid chromatography (GC) with electron capture detection was developed. This cost-effective method would allow mechanization and automation and would be faster and more accurate than previous pesticide residue procedures. A 10-mg hair sample, with added internal standard, in 1 mL of hexane was sonicated, vortexed, and centrifuged for 5 min during each step. Supernatant (1 μL) was injected on a DB-1 capillary column for GC analysis. Averaged recoveries of the permethrin on cattle hair were 95% for the cis isomer and 100.7% for the trans isomer. The on-column sensitivities were 10 pg for the cis isomer and 1 pg for the trans isomer. This method was used to determine the coverage and persistence of permethrin, applied by an electrostatic sprayer, on cattle hair coat.

Permethrin [(3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate] is one of the synthetic pyrethroids commonly used on livestock for pest management. Evaluations of insecticides for the protection of animals from biting flies are normally carried out in field trails at arbitrarily chosen doses. Efficacy is then determined by the fly counts on the treated animals (Lockwood et al., 1985; Hogsette and Ruff, 1986). Present field trials lack the ability to determine the actual delivered doses, the distribution pattern, and the persistence of the insecticide administered. It is conceivable that incorporation of residue analysis of hair samples with activities in field trials would optimize the dose and the application technique. This in turn should improve the safety and effectiveness of insecticide use.

Residue analysis of pyrethroids has recently been reviewed (Papadopoulou-Mourkidou, 1983; Sherma and Zweig, 1985; Ambrus and Thier, 1986; Hill, 1986; Sherma, 1987). Published analytical procedures for the determination of pyrethroid residues mainly use gas–liquid chromatography (GC) or high-performance liquid chromatography. Various GC methods for permethrin residue analysis in different matrices have been reported (Williams, 1976; Oehler, 1979; Carroll et al. 1981; Chapman et al., 1981; Reichel et al., 1981; Braun and Stanek, 1982; Marie et al., 1982; Hansen et al., 1983; Schimmel et al., 1983). Other workers have reported HPLC methods as alternatives to GC for analyzing permethrin (Kikta and Shierling, 1978; Lam and Grushka, 1978; Papadopoulou-Mourkidou et al., 1980, 1983; Rando and Hammad, 1985; Sundaram et al., 1985; Leidy et al., 1986), but none pertain to cattle hair. However, several reports discuss the quantitation of pyrethroids in cattle hair, but without detailed procedures (Taylor et al., 1985a,b, 1986; Kumar et al., 1986).

Traditional pesticide residue analysis requires a large sample size and a large volume of extraction solvent, followed by sample cleanup steps. Conversely, the electron capture detector requires only a small portion of the purified extract for injection into the GC for quantitation and is very sensitive and selective. With the ever-increasing sample load and cost of solvent and chemicals, these labor-intensive and uneconomical procedures should be reexamined.

The purpose of this study was to develop a simple, efficient, and cost-effective small-scale GC procedure for routine monitoring and quantitation of permethrin on cattle hair.

MATERIALS AND METHODS

Chemicals. Analytical standards of *cis*- and *trans*-permethrin and the internal standard (i.s.) *p*-chlorophenoxy analogue of *cis*-permethrin [3-[4-(chlorophenoxy)phenyl]methyl *cis*-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate] were synthesized in our laboratory according to Nakatsuka et al. (1977). The 3-(4-chlorophenoxy)benzyl alcohol was obtained by sodium borohydride reduction of 3-(4-chlorophenoxy)benzaldehyde (Yeung et al., 1982). The crude i.s. was purified by silica gel column chromatography. Elution with hexane (OmniSolv, BDH) afforded 45% yield of pure i.s. as a viscous colorless oil. All compounds were fully characterized by NMR, IR, and GC–MS, and purities (>99%) were checked by GC with flame ionization detection. The insecticide, Ectiban 25% emulsifiable concentrate (EC), formulated by Chipman Inc., Ontario, was used in this study. The ratio of *cis*- to *trans*-permethrin was found to be 45:55.

Insecticide Application. A dual-cloud electrostatic sprayer was used to apply 2 mL of Ectiban 25% EC in 500 mL of water onto a 451-kg steer. Weather conditions were overcast with a wind speed of about 10 km/h. The sprayer was mounted on the back of a truck stationed 7 m from the left rear of the tethered steer. The permethrin applied onto the steer was allowed to dry for 2 h before the steer was put into a barn for hair sampling.

Hair Sampling. Prior to permethrin spraying, 10 control hair samples, 25 cm^2 each, were collected from different representative areas of the steer with a livestock hair clipper. Hair samples were also taken at 0.1- (2-h), 4-, 7-, and 14-day intervals after spraying. Each time, 39 samples of 25 cm^2 each were collected: i.e., two sites from the head, four from the dewlap, nine from the back, three from each side, nine from the belly, two from each leg, and one from the tail. Each hair sample was individually wrapped in tin foil and put into a thick plastic bag. The clipper was thoroughly rinsed with methanol between each clipping to avoid cross contamination. The hair samples

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were then stored at −40 °C until analysis. Between hair sampling, the steer was isolated in an outdoor pen.

Extraction. The weights of 25-cm² hair samples ranged from 40 to 600 mg. A subsample of 10 mg of hair was weighed into a culture tube, and 0.5 μg (20 μL) of i.s. was added. The hair samples were extracted with 1 mL of hexane in an ultrasonic bath (Branson) for 5 min. The tubes were then vortexed (Vibrax) for another 5 min and centrifuged (Silencer) at 2400 rpm for 5 min. The supernatant was transferred to sample vials. A 1-μL sample was injected by an autosampler on a DB-1 capillary column for GC analysis.

Standard Curves. The standard curves were prepared by spiking 10 mg of untreated hair with authentic standards at eight increments (0, 0.01, 0.05, 0.1, 0.5, 1, 2, 3 μg) and a fixed quantity of i.s. (0.5 μg). These hair samples were extracted and anayzed along with extracts from treated and untreated hair samples.

Recoveries. The recovery study was carried out by fortifying 10 mg of the untreated cattle hair with permethrin at levels of 0.1, 0.5, 1, and 1.5 μg along with 0.5 μg of i.s. The samples were air-dried for 2 h and stored at −40 °C for 24 h before the entire analytical procedure was performed.

Quantitation. The calibration curves were constructed by plotting the ratio of peak areas of *cis*- and *trans*-permethrin to peak areas of i.s. against the respective concentrations. The amount of *cis*- and *trans*-permethrin in the treated hair samples was calculated by least-squares linear regression analysis. For quantitation and data analysis, the statistical analysis system (SAS) program (SAS Institute Inc., 1985) was used in a VAX 11/750 computer.

Instruments. A HP 5890A GC equipped with a 15-mCi ^{63}Ni source electron capture detector, 7673A autosampler, and 3292A integrator was used. The capillary column used was a DB-1 fused silica column, 15 m × 0.25 mm (i.d.) (J & W Scientific). Helium at 2 mL/min was used as carrier gas, and argon–methane (95:5) at 61 mL/min was used as makeup gas. Head pressure was maintained at 10 psi. The following oven program was employed: Initial temperature of 170 °C was maintained for 0.6 min and was increased at a rate of 20 °C/min to 260 °C. The temperature was increased again at a rate of 3 °C/min to 270 °C and was maintained at this temperature for 4 min. The temperature of the injection port was 230 °C, and that of the detector was 300 °C. Injection volume (splitless) was 1 μL. Gas chromatography–mass spectrometry (GC–MS) was conducted using a HP 5985B spectrometer to obtain confirmatory electron impact spectra.

RESULTS AND DISCUSSION

Analytical Method. Typical chromatograms obtained from GC with electron capture detection of spiked, treated, and control cattle hair samples are shown in Figure 1. The chromatograms were clean and with no interfering peaks, even without a cleanup step. The *cis*- and *trans*-permethrin were reasonably well resolved within 12-min run time. Structures of the permethrin and i.s. in a treated cattle hair sample were confirmed by GC–MS. Mass spectral data were isolated from agreement with that reported by Reichel et al. (1981).

Calibration linearity (0.01–3 μg) for both *cis*- and *trans*-permethrin was excellent, with correlation coefficients of >0.99 obtained routinely. The recoveries of these isomers were good, with an average of 95% for *cis*-permethrin and 100.7% for *trans*-permethrin (Table I). Standard curves prepared with or without hair gave the same quantitations in the recovery study. The on-column

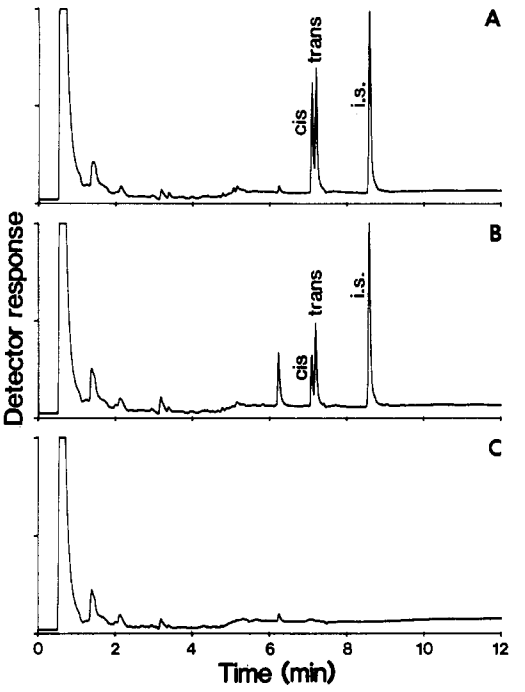

Figure 1. Typical GC chromatograms for the determination of permethrin on cattle hair: (A) extract of control cattle hair sample spiked with 0.5 μg each of *cis*- and *trans*-permethrin and i.s.; (B) extract of treated hair sample; (C) extract of control untreated cattle hair sample.

Table I. Recoveries of *cis*- and *trans*-Permethrin from Fortified Cattle Hair

	recovery,[a] %	
spiked,[b] μg	cis	trans
0.1	107 (2.9)	104 (5.7)
0.5	100 (4.4)	101 (5.5)
1.0	89 (2.8)	101 (3.6)
1.5	85 (1.8)	97 (2.7)

[a] Mean value (SD), $n = 4$. [b] Untreated 10-mg cattle hair samples were fortified with 1:1 *cis*- to *trans*-permethrin.

sensitivities were 10 pg for the cis and 1 pg for the trans isomer, with a signal to noise ratio of at least 5:1. Using this cost-effective procedure, all precautions mandated for pesticide residue analysis (Fehringer and Walters, 1986) were observed.

The growing demand for pesticide residue monitoring in livestock, crop, and enviromental samples means that an ever-increasing number of samples require analysis. Traditional methods of analysis usually involve large sample size and large volume of extraction solvent followed by sample cleanup steps (Williams, 1976; Oehler, 1979; Carroll et al., 1981; Chapman et al., 1981; Reichel et al. 1981; Braun and Stanek, 1982; Marie et al., 1982; Hansen et al., 1983; Schimmel et al., 1983). With the advent of capillary column and electron capture detection, only a small portion (usually 1–2 μL) of the purified extract is injected in a GC for quantitation. It is not logical to clean up large sample volumes just for disposal.

The use of an internal standard in pesticide residue analysis has not yet been commonly accepted because internal standards are not commercially available and need to be synthesized. The *p*-Cl analogue of *cis*-permethrin was therefore synthesized as an appropriate i.s. for this assay. There should be little question that the use of an i.s. will improve the precision and accuracy of a chromatographic assay (Marie et al., 1982; Hill, 1986). To handle a small volume of extraction solvent (1–2 mL), the use of

Table II. Distribution and Persistence of Permethrin on the Hair Coat of a Steer Treated with 2 mL of 25% Ectiban EC Applied by a Dual-Cloud Electrostatic Sprayer

location	no. of samples	amount,[a] μg/g 0.1 day cis	0.1 day trans	4 days cis	4 days trans	7 days cis	7 days trans	14 days cis	14 days trans
head	2	7.7 (2.2)	9.3 (2.9)	5.8 (0.5)	9.9 (3.0)	6.5 (0.4)	9.1 (0.5)	6.6 (0.8)	6.9 (1.9)
dewlap	4	6.9 (1.0)	7.8 (1.4)	5.9 (0.4)	7.3 (1.1)	5.9 (0.5)	7.3 (1.0)	5.3 (0.3)	5.8 (0.8)
back	9	33.1 (20.1)	42.6 (26.1)	11.4 (8.4)	14.2 (10.8)	7.2 (1.7)	9.1 (1.9)	5.9 (0.5)	6.1 (0.5)
side	6	43.6 (44.3)	33.7 (32.8)	7.6 (2.4)	10.0 (3.4)	7.1 (0.8)	9.4 (1.2)	5.6 (0.5)	6.1 (0.7)
belly	9	13.1 (13.0)	15.8 (17.0)	6.0 (1.1)	8.4 (2.9)	5.8 (0.4)	7.1 (3.6)	5.5 (0.2)	5.5 (0.2)
legs	8	10.8 (3.9)	13.5 (5.1)	6.8 (0.8)	8.6 (0.9)	6.2 (0.5)	8.0 (1.0)	5.8 (0.8)	5.8 (0.3)
tail	1	68.1	88.4	8.4	10.6	7.0	9.9	5.0	5.5

[a] Mean value (SD).

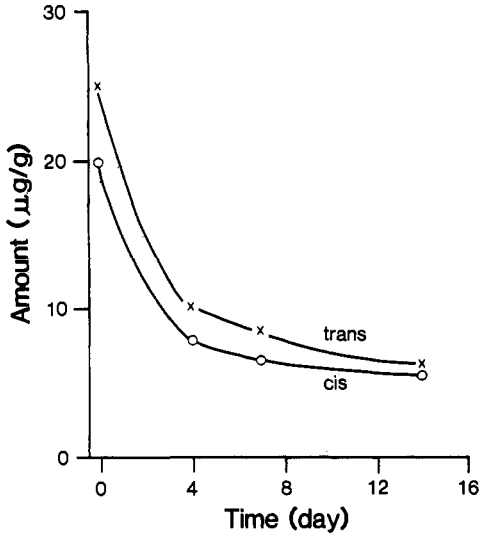

Figure 2. Dissipation of *cis*- and *trans*-permethrin on the treated hair coat of a steer. Each value is a mean of all 39 sites sampled on the steer.

i.s. is deemed necessary (Ramsteiner, 1986).

We have developed a simple, small-scale permethrin analytical procedure with low running cost suitable for routine analysis of a large sample load. Reduction in the scale of analysis opens new ways of mechanization or automation of residue analysis (Forbes and Dutton, 1985; Ramsteiner, 1986). This small-scale approach is particularly useful in long-term studies, such as the persistence of insecticide on cattle hair coat from insecticide-impregnated ear tags. About 10–20 mg of hair can be sampled from each site. This way, the hair coat of the cattle will not be altered significantly by hair samplings throughout the pest season.

Permethrin Levels on Cattle Hair. Initial residues on cattle hair collected from the 39 sites on the steer indicated that the electrostatic sprayer was capable of providing a total body coverage of cattle (Table II). At 0.1 day (2 h) after spraying, the average amount of total permethrin (*cis*- and *trans*-permethrin) loaded onto the steer was 45 μg/g of hair. Areas closer to the sprayer, such as the tail, the back, and the left side, received a higher dose of permethrin, up to 160–180 μg/g of hair. The amount of permethrin on other areas was relatively even as indicated by the standard deviations in Table II. This uneven distribution of permethrin was no longer apparent from day 4 onward. The data showed that some of the areas had an equal or slightly higher amount of permethrin on day 4 than on day 0.1. This indicated that a redistribution process was occurring, probably due to self-grooming activities.

The persistence of permethrin in cattle hair is summarized in Figure 2. Slopes in semilog plot did not show any appreciable differences in dissipation rates of *cis*- and *trans*-permethrin up to 7 days. This finding was different from that reported on the metabolism of permethrin in animals and soil where the trans isomer was metabolized faster than the cis isomer (Gaugham et al., 1978; Ivie and Hunt, 1980; Jordan et al., 1982; Marie et al., 1982; Jordan and Kaufman, 1986). The reason for the lack of differences in our study is not known. Perhaps the process of dissipation of permethrin on cattle hair coat did not significantly involve microbial metabolism, which was the case in permethrin-treated soil (Jordan et al., 1982; Jordan and Kaufman, 1986).

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Registry No. Permethrin, 52645-53-1; *cis*-permethrin, 61949-76-6; *trans*-permethrin, 61949-77-7.

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