ETOC™ AND LAMBDACYHALOTHRIN: NEW PYRETHROID MOSQUITO ADULTICIDES

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ABSTRACT. Two new pyrethroids, ETOC™ and lambdacyhalothrin, showed considerable promise against organophosphorus-resistant adults of *Culex tarsalis* in laboratory tests. In field trials using nonthermal aerosols, applications of both compounds resulted in effective swaths of up to one-half mile. At the highest concentrations evaluated, residues on alfalfa foliage were less than 0.2 ppm at 1 h. Residues declined greatly by 24 h and could not be detected at 48 or 72 h.

INTRODUCTION

The number of adulticides available to California mosquito abatement agencies has been reduced due to insecticide resistance, e.g., malathion, and to removal of products from the marketplace, e.g., propoxur and fenthion, rather than going through the expensive process of reregistration. The need for safe and effective adulticides was demonstrated by an outbreak of St. Louis encephalitis (SLE) in the San Joaquin Valley during 1989; efforts to suppress transmission of SLE by *Culex* species were not successful, and there remains an urgent need to obtain new adulticides (Reisen et al. 1990). Two new pyrethroid insecticides were obtained for evaluation against mosquito adults during 1989: ETOC™ (2 methyl-4-oxo-3-(2-propynyl) cyclopent-2-enyl chrysanthemate) and lambdacyhalothrin (alpha-cyano-3-phenoxybenzyl-(2 chloro-3,3,3-trifluoroprop-1-enyl)-2-2-dimethylcyclopropanecarboxylate). This paper summarizes laboratory and field evaluations as well as preliminary studies of their residues on vegetation.

MATERIALS AND METHODS

Insecticides: The structures of both compounds are shown in Fig. 1. Samples of technical material were provided by the respective manufacturers (Sumitomo Chemical America, New York, NY, for ETOC and ICI Americas, Goldsboro, NC, for lambdacyhalothrin). For field tests, a 2% oil solution of lambdacyhalothrin was diluted with a mineral oil (Orchex 796) to achieve the desired concentration. Two oil formulations of ETOC were evaluated: 1) a 0.6% (AI) solution also containing 1.8% piperonyl butoxide (PBO), and 2) a 2.46% (AI) solution containing 7.38% PBO; the first formulation was applied directly and the second was diluted with Orchex 796 mineral oil.

Laboratory studies against adult mosquitoes: Glass filter papers (9 cm Whatman GF/A) were treated with a series of concentrations of technical insecticide in acetone (Georghiou and Gidden 1965). The papers were rolled and placed into vials in which *Culex tarsalis* Coq. adults were exposed. Approximately 20 adults of a given strain were placed in the vials for a 1 h exposure period and then transferred into untreated cups for mortality determination at 24 h after treatments. For ETOC, 5 concentrations were tested against the susceptible strain (range 0.003–0.015% (AI) solution) and 7 concentrations (range 0.002–0.02% (AI) solution) against the organophosphorus (OP)-resistant strain. For lambdacyhalothrin, 7 concentrations were tested against the susceptible (range 0.0001–0.003% (AI) solution) and 7 against the OP-resistant strain (range 0.0001–0.01% (AI) solution). Each concentration was made in duplicate for each test; each test was triplicated. Mortality data were corrected for control mortality using Abbott's formula (Abbott 1925). Data were analyzed using SAS probit analysis (SAS Institute 1985).

Field evaluations using nonthermal aerosol fogging: Field tests were conducted in remote desert locations where no crops or livestock were present. A Micro-Gen Model G-9HD (Micro-Gen Equip. Corp., San Antonio, TX) mounted in the bed of a pickup truck was used for all applications. The truck was calibrated and driven at 5 mph and the aerosol generator calibrated to deliver 9 fluid ounces per minute. Spray particle size was monitored by exposing teflon-coated slides, measuring the droplet sizes under high magnification and then plotting droplet diameter versus cumulative volume to determine the volume mass diameter (Carroll and Bourg 1979).

Wind and temperature conditions were monitored using a R. M. Young Model 41402-J recording weather station (R. M. Young Company, Traverse City, MI). Temperatures were recorded at 8 and 32 ft, and wind direction and velocity at 15 ft. A temperature inversion of at least 0.3°C and wind within the range of 2–8 mph were required for every application.

Efficacy of the aerosol was monitored using caged *Cx. tarsalis* adults placed on 4 ft high

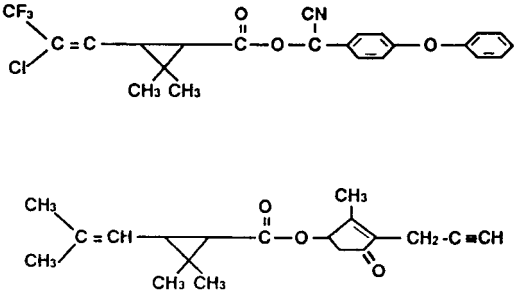

Fig. 1. Structures of lambdacyhalothrin (above) and ETOC (below).

stakes in duplicate lines with cages at distances of 100, 200, 300, 400, 500, 600, 1,320 and 2,640 ft downwind of the application path. Mortalities were recorded at 1 h posttreatment in the field and at 14 h after transporting back to the laboratory. Control cages were located upwind of the application line. The effective swath width was defined to be the distance downwind in which final mortality averaged 90% or greater. Test size and replications were restricted to comply with the EPA limit of 10 acres per chemical without an Experimental Use Permit.

For the test involving the highest application concentration of each compound, a row of potted alfalfa plants was placed 150 ft downwind of the application line, and duplicate samples of treated plants were taken at 1, 24, 48 and 72 h after treatment. Control potted plants were placed upwind of the application path and were sampled, in duplicate, as per treated plants.

Analytical methods: An analytical method was developed to allow the quantitative determination of each compound on alfalfa foliage.

Extraction: Twenty g of foliage was placed in a Sorval omni-mixer with 40 g anhydrous Na_2SO_4. For ETOC, 100 ml hexane was added; for lambdacyhalothrin, 100 ml acetonitrile was added. The mixture was blended for 5 min and then filtered through Whatman no. 42 paper and then through a Millipore (Millex-SR 0.5 μm) filter into a stoppered flask.

Liquid partition cleanup: A 50 ml aliquot of the ETOC in hexane (above) was partitioned twice against 50 ml acetonitrile (saturated with hexane); the combined acetonitrile phase was reduced to dryness and then dissolved in 2–3 ml hexane. A 50 ml aliquot of the lambdacyhalothrin in acetonitrile (above) was partitioned twice against 50 ml of hexane (saturated with acetonitrile); the acetonitrile phase was reduced to dryness and then dissolved in 2–3 ml of hexane.

NH_2 column cleanup: The above samples were then placed on a column (1 × 20 cm) containing 2 g Sepralyte-NH_2 sorbent (Analytichem International, Harbor City, CA). For ETOC, the column was first eluted with 50 ml hexane, which was discarded, and the active ingredient was then eluted with 10 ml ethyl acetate/hexane (1:3 v/v); the latter was taken to dryness and the sample was dissolved in 2–3 ml hexane for cleanup on Florisil. For lambdacyhalothrin, the sample from the previous cleanup was added to the column and the active ingredient was eluted with 100 ml hexane, which was then reduced to 2–3 ml for cleanup on Florisil.

Florisil cleanup: A glass column (1 × 20 cm) was packed with 5 g Florisil and samples from the previous step were added. For ETOC, the column was eluted with 10 ml hexane and then 25 ml of ethyl acetate/hexane (4:96 v/v); these were discarded and the active ingredient was eluted with 40 ml ethyl acetate/hexane (10:90 v/v). The volume was then reduced to dryness and the residue dissolved in 1 ml acetonitrile for analysis by high performance liquid chromatography (HPLC). For lambdacyhalothrin the sample was followed by 25 ml hexane, which was discarded and the active ingredient was eluted with 50 ml ethyl acetate/hexane (1:3 v/v). The latter fraction was reduced to dryness and the residues were dissolved in 1 ml hexane for analysis by gas-liquid chromatography (GLC).

HPLC: A Perkin-Elmer model 400 instrument equipped with a UV/visible detector was used for reversed-phase analysis. The C-18 column (4.6 × 83 mm) was eluted with acetonitrile/water (60:40 v/v) at 1 ml/min while held at 40°C. The spectrophotometer was set at 220 nm. The data were sent to a computing integrator.

GLC: A Hewlett-Packard model 5890 gas-chromatograph was equipped with an electron capture detector. The column was 0.2 mm × 12 m cross-linked methyl silicone. Operating temperatures were: column 200°C for 10 min then 20°C/min to 230°C, injection port 280°C, detector 300°C. The helium carrier gas was delivered at 0.67 ml/min through the column and 20 ml/min through the flow-vent; the make-up gas was argon/methane (95%/5%) delivered at 60 ml/min.

Confirmation of active ingredient: To confirm the presence of the active ingredient, samples were subjected to GLC-mass spectrometry (GLC-MS). A Hewlett-Packard model 5988 mass spectrometer was operated in the electron impact mode at 70 EV. For lambdacyhalothrin, the GLC conditions were as above. For ETOC, the column and carrier gas were as above with the following temperatures: column 190°, injection port 250° and transfer line 200°C. To analyze the amounts present in the residue samples, selective ion monitoring was used. Nine ions were monitored for each compound: for ETOC, M/Z 77, 79, 81, 103, 105, 123, 134, 168 and the molecular ion at M/Z 300; and for lambdacyhal-

othrin, M/Z 77, 115, 141, 181, 197, 199, 208, 209 and the molecular ion at M/Z 449.

Method validation: To confirm the validity of the methods described, triplicate samples of alfalfa were fortified using technical standards of each compound at 10.0, 1.0, 0.1, and 0.01 ppm and extracted and analyzed as above.

RESULTS AND DISCUSSION

Laboratory tests: Some of the adults that survived the exposures had lost several legs but were still able to stand upright and to fly. Adults able to do so were scored as live. The loss of legs in pyrethroid-treated adults has been reported previously (Khoo and Sutherland 1981, Liu et al. 1986). Both compounds show very high activity but lambdacyhalothrin is about 1 log-fold more active than ETOC (Table 1). The LC_{95} values were about 2-fold greater for the OP-resistant strain than for the susceptible strain for both compounds, and the slopes for the OP-resistant strain were much lower. We do not regard this as an indication of cross-resistance, as there was less than a 5-fold difference in the LC_{95} values between strains. Such difference between strains was described as possible "vigor tolerance" by Hoskins and Gordon (1956).

It is important to obtain laboratory baseline susceptibility data on field populations of mosquitoes in a given area prior to initiating the use of pyrethroids. Cross-resistance to pyrethroids in DDT-resistant strains has been shown in both *Aedes* and *Anopheles* species (Chadwick et al. 1977, Prasittisuk and Busvine 1977) as well as in OP-resistant *Culex* (Priester and Georghiou 1978, 1979).

Field efficacy trials: Numerous attempts (ca. 24) to conduct the nonthermal aerosol tests had to be aborted because of unsatisfactory meteorological conditions (lack of steady wind in the 2–8 mph range or lack of a temperature inversion). However, 7 tests were successfully conducted and are summarized in Table 2.

Using the 0.6% (AI) formulation of ETOC (undiluted) resulted in larger droplet sizes than desired (volume mass diameter (VMD) 23 μm). The following 2 trials used a more concentrated (2.46% AI) formulation, which was diluted with Orchex 796 mineral oil and resulted in a smaller droplet spectrum (VMD, 17 μm). In both trials using 0.6% ETOC, there were scattered survivors in the cages, indicating that the concentration of AI in the aerosol should not be further reduced. Using the 1.2% concentration of ETOC the efficacy was limited to 600 ft downwind due to a crosswind effect.

All lambdacyhalothrin applications were conducted with the 2% formulation diluted with Orchex 796, and this resulted in a consistent VMD of 15 μm. The last application (1% AI) was applied under near perfect meteorological conditions and resulted in 90% or greater mortality in all of the cages. For the 3 lower concentrations, the aerosol fog contained sufficient toxicity for operational success and the effective

Table 2. Summary of 1989 applications of lambdacyhalothrin and ETOC against caged *Culex tarsalis* adults.

% AI in aerosol	Effective swath (ft)[a]	Application rate (lb (AI)/9 oz)	Dose actually applied[b] (lb (AI)/acre)
		ETOC	
0.6[c]	1,320	0.0030	0.00023
0.6[d]	2,640	0.0030	0.00011
1.2[d]	600	0.0015	0.0054
		Lambdacyhalothrin	
0.5	500	0.0029	0.00062
0.25	600	0.0015	0.00027
0.125	1,320	0.00073	0.000056
1.0	2,640	0.0059	0.00021

[a] Average mortality of over 90%.
[b] Calculated based on the effective swath width.
[c] 0.6% AI formulation containing 1.8% piperonyl butoxide.
[d] 2.46% AI formulation containing 7.38% piperonyl butoxide.

Table 1. Mortality of *Culex tarsalis* adults exposed to filter papers treated with ETOC or lambdacyhalothrin.[a]

LC_{50}	95% confidence limits	LC_{95}	95% confidence limits	Slope and standard error
		ETOC—susceptible strain		
0.0070	0.0061–0.0081	0.013	0.011–0.018	6.00 ± 0.74
		ETOC—OP-resistant strain		
0.0068	0.0046–0.0102	0.020	0.013–0.088	3.43 ± 0.73
		Lambdacyhalothrin—susceptible strain		
0.00059	0.00046–0.00077	0.0029	0.0019–0.0060	2.37 ± 0.28
		Lambdacyhalothrin—OP-resistant strain		
0.00057	0.00049–0.00066	0.0063	0.0047–0.0090	1.58 ± 0.10

[a] % solution applied to filter paper.

Table 3. Recoveries of ETOC and lambdacyhalothrin from untreated alfalfa samples fortified at 4 levels and analyzed in triplicate.

Level fortified (ppm)	Found (ppm) Mean ± SD	% recovery Mean ± SD
	ETOC	
10.00	9.35 ± 0.38	93.47 ± 3.87
1.00	0.95 ± 0.011	95.31 ± 1.13
0.10	0.090 ± 0.0031	89.35 ± 3.05
0.01	0.0093 ± 0.00031	92.94 ± 3.13
	Lambdacyhalothrin	
10.00	9.10 ± 0.32	91.01 ± 3.18
1.00	0.95 ± 0.034	95.35 ± 3.44
0.10	0.093 ± 0.0047	93.19 ± 4.74
0.01	0.0092 ± 0.00067	91.55 ± 6.65

swath distances were determined by meterological conditions.

Both ETOC and lambdacyhalothrin offer high potential for "aerosol applications" when suitable meteorological conditions occur. Additional studies using aircraft applications are needed. Such tests were not feasible in 1989 due to the small amounts of formulations available and to the lack of EPA Experimental Use Permits. However, these results now provide justification for conducting additional trials.

Chemical residue occurrence and persistence: The recovery of ETOC and lambdacyhalothrin from alfalfa foliage fortified at 4 different levels is presented in Table 3. It is clear that the methodology was suitable for determining presence of the compounds, on alfalfa foliage in the 0.01–10.0 ppm range. Using these methods, it was estimated that the lowest amount that could be detected (minimum acceptable chromatographic peak of twice background level) was 0.0013 ppm for ETOC and 0.0014 ppm for lambdacyhalothrin.

No residues (<0.0013 ppm) of either compound were detected on alfalfa of the controls or from plants sampled at 48 h or 72 h after treatment. At 1 h and at 24 h, residues were 0.19 ± 0.00025 ppm and 0.034 ± 0.0024 ppm for ETOC and 0.14 ± 0.022 ppm and 0.082 ± 0.012 ppm for lambdacyhalothrin. The identities of the active ingredients found in these samples were confirmed by GLC-MS. Thus, the residues substantially declined between 1 and 24 h and had totally dissipated by 48 h. Because these residues resulted from the highest concentrations applied (1.23% AI for ETOC and 1.0% AI for lambdacyhalothrin), no evidence of an unfavorable chemical persistence problem on foliage was apparent for either compound.

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