region; however, it seems affected by interferences from beeswax to a greater extent.

Registry No. Phenol, 108-95-2.

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Insecticidal Activity of Tralomethrin: Electrophysiological Assay Reveals That It Acts as a Propesticide

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The activity of the pyrethroid tralomethrin was assessed by using an electrophysiological assay (monitoring the release of miniature excitatory postsynaptic potentials) on the larvae of *Heliothis virescens*, *Chilo partellus*, and *Plutella xylostella*. Small quantities of deltamethrin in the tralomethrin sample were sufficient to explain the observed activity. Tralomethrin is shown to be unstable and debromination of tralomethrin to deltamethrin occurred rapidly in simulated sunlight. There is therefore evidence that tralomethrin is not intrinsically active.

Tralomethrin [HAG 107: (*S*)-α-cyano-3-phenoxybenzyl (1′*RS*)-*cis*-(1*R*,3*S*)-3-(1,2,2,2-tetrabromoethyl)-2,2-dimethylcyclopropanecarboxylate] is known to be unstable, decomposing to deltamethrin [NRDC 161: (*S*)-α-cyano-3-phenoxybenzyl *cis*-(1*R*,3*R*)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate] in ultraviolet light (Ruzo and Casida, 1981), on topical application to houseflies, and on feeding to cabbage looper larvae (Ruzo et al., 1981).

Despite the instability of tralomethrin, it is not known whether its insecticidal activity is due to decomposition to deltamethrin or whether it possesses insecticidal activity in its own right. To find out if tralomethrin is intrinsically active, the pyrethroid must be tested at its site of action in the insect. Only by this method can reliable information be obtained without the complicating factors of penetration and metabolism.

Pyrethroid insecticides kill insects by affecting the nervous system (Gammon et al., 1982). Within the nervous system, motor nerve terminals have been identified as particularly susceptible to disruption by pyrethroids and their disruption correlates with pyrethroid poisoning in vivo (Adams and Miller, 1979; Miller et al., 1983; Miller and Adams, 1982; Narahashi and Lund, 1980; Salgado, 1981; Salgado et al., 1983; Omer et al., 1980). The onset and degree of pyrethroid poisoning can be assayed by monitoring the change in function of a process that is under nerve terminal control. The rate of release of miniature excitatory postsynaptic potentials (mepps) is such a process—indeed, the first sign of pyrethroid poisoning in vitro is a vast increase in the frequency of mepps. By increasing the concentration of pyrethroid until an increase in mepp frequency is observed, a reproducible measure of the intrinsic activity of a pyrethroid can be obtained. Such data, in a series where differences of penetration and metabolism can be ignored, give a good correlation between in vivo and in vitro potency (Miller and Adams, 1982; Miller et al., 1983; Salgado, 1981; Salgado et al., 1983; Irving, 1983). Some previous electrophysiological assays on pyrethroids have yielded a poor correlation between in vivo and in vitro potency [e.g., Narahashi et al. (1977)] probably because the tissue used (nerve cord, in this case of crayfish) is not the primary site of action of pyrethroids, necessitating the use of excessive concentrations.

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MATERIALS AND METHODS

Chemical Analysis. Tralomethrin and deltamethrin were analyzed by high-performance liquid chromatography (HPLC). The system used consisted of a Waters Associates 6000A pump, an Altex Model 210 injection valve, and a Pye Unicam LC–UV detector. Columns used were 100 mm × 4.6 mm i.d., and all solvents were prefiltered and of HPLC or glass distilled grade. Two contrasting separation systems were used, both giving base-line separation of deltamethrin and the two isomers of tralomethrin: system A, Spherisorb S5ODS (Chrompack), mobile phase 85% methanol/15% water, flow rate 1 mL/min, and analytical wavelength 230 nm; system B, Spherisorb S5W silica (Chrompack), mobile phase 60% hexane/40% dichloromethane, flow rate 1 mL/min, and analytical wavelength 240 nm. Concentrations of deltamethrin and tralomethrin were measured by direct comparison with standards of similar concentration.

Photochemistry. Tralomethrin (formulated as a 3.75% emulsifiable concentrate containing a red azo dye (to act as a filter for ultraviolet light) was irradiated as thin films (0.75 μg of active ingredient/cm^2) on glass under simulated sunlight. The lamp array used (6 × 40 W Thorn UV fluorescent tubes, 20 cm above the sample plates) has been shown to be 2–5 times less intense than British summer sunlight in its ability to photodegrade a variety of pesticides (Fraser, 1983).

Electrophysiological Assay. Mepp release was monitored by using the fourth instar larvae of the lepidopterans *Heliothis virescens*, *Chilo partellus*, and *Plutella xylostella*. The larvae were dissected by dorsal incision and the gut/viscera/fat removed to expose the nervous system and musculature. The preparations were flooded with saline

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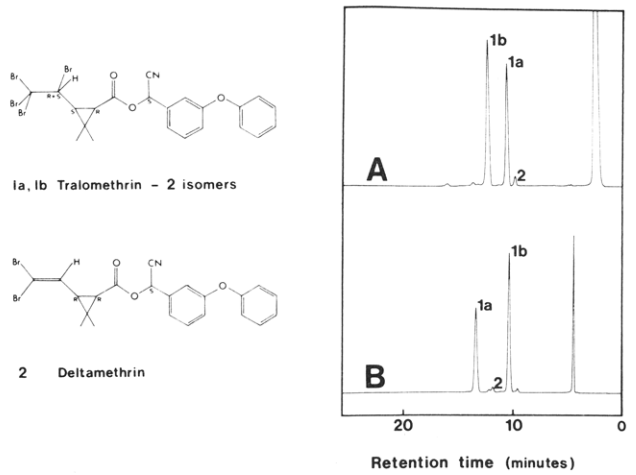

Figure 1. Separation of tralomethrin and deltamethrin by HPLC. (A) Spherisorb ODS; 85% methanol/15% water; 1 mL/min; 230 nm. (B) Spherisorb S5W silica; 60% hexane/40% dichloromethane; 1 mL/min; 240 nm.

[(Weevers, 1966) for *Chilo/Plutella* and Weevers saline plus 150 mM sucrose added for *Heliothis*]. Muscles used for the assay were the longitudinal muscles (intersegmental, 1, 3, and 5; Belton, 1969). Muscle fibers were impaled with 3 M KCl filled electrodes (10–15 MΩ) and miniature release was monitored by using conventional low-noise recording techniques. Intracellular measurement of miniature rate is less precise than extracellular focal recording at the synapse (see Figure 2D), being prone to changes in space constant for fiber to fiber. However, the buried position of lepidopteran nerve terminals within the muscle (Rheuben and Reese, 1978) makes such focal recordings very difficult and impractical for any routine assay system. The increase in mepp frequency was sufficiently great, however, to make the identification of poisoned units by intracellular recording unmistakable.

Stock solutions of pyrethroid in AnalaR acetone were made up immediately prior to testing to minimize any decomposition that might occur before the bioassay. The pyrethroids were then diluted in saline to the required concentration and added to the preparation. The preparation was left to equilibrate with the pyrethroid for 45 min. Calibration of the assay with radiolabeled cypermethrin has shown that following the dilution regime and incubation, 85% of the calculated pyrethroid is still present in solution; the 15% loss is presumably due to adsorption onto glass and wax surfaces. Following incubation for the time stated, the longitudinal fibers were sampled and the number of fibers showing increased mepp frequency was noted. The pyrethroid-induced increase in mepp frequency is sufficiently large that muscle fibers innervated by affected nerve terminals are unmistakeable (see Figure 2). Dissected preparations exhibit very little spontaneous motor activity; any motor activity recorded during the assay was therefore attributed to pyrethroid-induced antidromic firing and taken as indicative of poisoning (Adams and Miller, 1979). To reduce the risk of light-induced pyrethroid decomposition, all pyrethoid dilutions and incubations were undertaken in subdued light.

Double-blind experiments on *Heliothis* (three larvae per pyrethroid concentration, 34 fibers/larva sampled) and *Chilo* (four larvae per pyrethroid concentration, 30 fibers/larva sampled) used pyrethroid samples coded with random numbers generated by computer. The *Plutella* experiment used the same four larvae and measured mepp release after a 45-min incubation in increasing pyrethroid concentration. The number of fibers per larva used in the

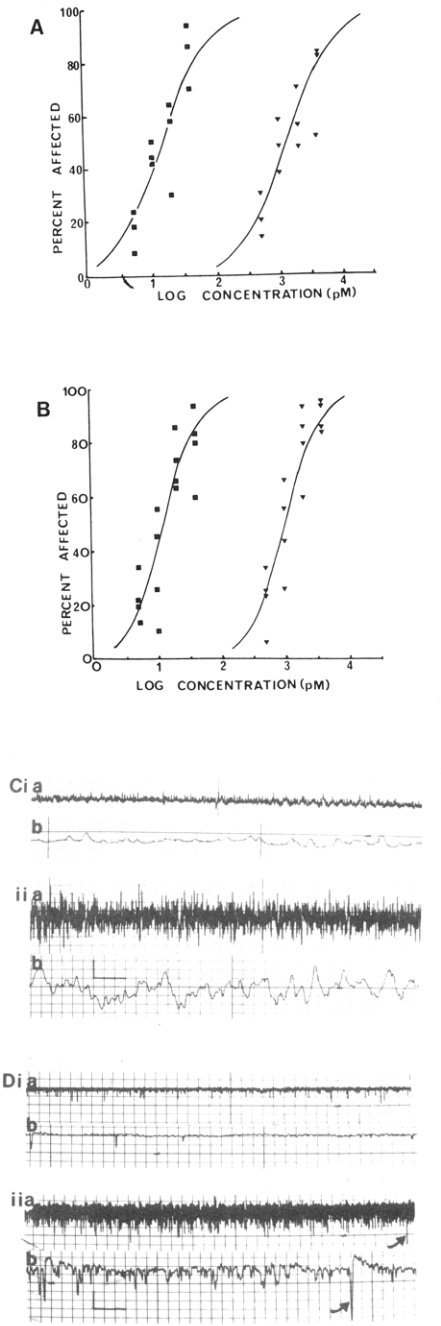

Figure 2. (A) Dose–response curve for deltamethrin and tralomethrin on muscle fibers of *Heliothis* larvae. Each point represents the percent of fibers affected of the 34 fibers sampled in each larva. Squares = deltamethrin; triangles = tralomethrin. Curves fitted by logit analysis. The potency ratio is 0.0104 (95% confidence limits: 0.0159–0.0068). The assay temperature = 18 °C. (B) Dose–response curve for deltamethrin and tralomethrin on muscle fibers of *Chilo* larvae. Each point represents the percent of fibers affected of the 30 fibers sampled in each larva. Squares = deltamethrin; triangles = tralomethrin. Curves fitted by logit analysis. The potency ratio is 0.0133 (95% confidence limits: 0.0185–0.0095). The assay temperature = 18 °C. (C) Miniature excitatory postsynaptic potentials (mepps) recorded intracellularly from longitudinal muscle fibers of *Heliothis* larva: (Ci,a,b) normal mepps on different time bases. (Cii,a,b) Mepps recorded from the same fiber as (Ci) after 45 min in 10 pM deltamethrin. Calibration: 1 mV; 3 s (a); 600 ms (b). (D) Mepps recorded extracellularly by focal recording (NaCl filled, 5 MΩ) from longitudinal muscle fibers of *Plutella* larva: (Di,a,b) extracellularly recorded mepps on two time bases. (dii,a,b) Extracellularly recorded mepps from the same synapses as in (Di) after 45 min in 10 pM deltamethrin. Arrows show epsp produced by the axon firing. Calibration: 500 mV; 3 s (a); 600 ms (b).

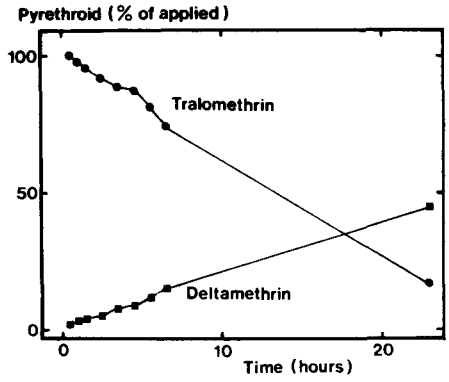

Figure 3. Conversion of tralomethrin to deltamethrin on irradiation with ultraviolet light (see the text for details).

Plutella assay was variable but greater than 22 fibers/larva. This was due to the topography of the fat, which obscured some fibers.

RESULTS

By use of HPLC analysis (Figure 1 shows typical chromatograms) the sample of tralomethrin used for bioassay was found to contain ca. 2.0% deltamethrin (in molar terms). Attempts to obtain a sample of pure tralomethrin by preparative HPLC were only partially successful due to further breakdown.

Bioassay results are shown in Figure 2. The activity of tralomethrin was consistently less than that of deltamethrin, to such an extent that the hypothesis was proposed that the activity of tralomethrin is due to its contamination with deltamethrin. If this is the case, then the slopes of the two dose–response curves should be parallel. Statistical analysis showed that in each case the null hypothesis that the two products have parallel dose–response curves is entirely plausible. The results indicate that tralomethrin exhibited the following percentage of the activity of deltamethrin: for *Heliothis*, 1% (95% confidence limits 0.7–1.6%; slope of logit response against log dose = 2.7); for *Chilo*, 1.3% (95% confidence limits 0.9–1.8%; slope of logit response against log dose = 3.6); for *Plutella*, 1.1% (95% confidence limits 0.9–1.3%; slope of logit response against log dose = 9.3). Data for Plutella are not shown in Figure 2.

It should be noted that parallel dose–response curves are expected from the same compound at different concentrations. However, as tralomethrin may fortuitously give a dose–response curve parallel to that of deltamethrin, this situation should not be taken as evidence that tralomethrin is intrinsically inactive.

Figure 3 illustrates the conversion of tralomethrin to deltamethrin on ultraviolet irradiation of thin films on glass plates. After 22.5 h of irradiation, only 17% of the tralomethrin remained, and deltamethrin accounted for 44% of the applied pyrethroid. Further (unidentified) products and vapor losses explain the poor recovery. Concentrations used were somewhat higher than recommended field rates (0.0156 lb of a.i./acre = 0.17 $\mu g/cm^2$), and the ultraviolet intensity of the lamps used was significantly weaker than British summer sunlight. The rate of conversion under field conditions should therefore be similar to or greater than that observed in this experiment.

DISCUSSION

The bioassay data indicate that the tralomethrin sample used, containing ca. 2.0% deltamethrin, gave 1.0% (1.6–0.7%), 1.3% (1.8–0.7%), and 1.1% (1.3–0.9%) of the activity of deltamethrin (see Results and Figure 2). This analysis is based on the premise that if the activity of tralomethrin and deltamethrin were caused by the same compound, their dose–response curves would be parallel (see discussion of this point under Results). The quantity of deltamethrin contaminating the tralomethrin sample is therefore sufficient to account for all the observed pyrethroid activity. This conclusion is strengthened by the fact that the data were obtained from three different species and three different slopes of the dose–response curve. There is thus no evidence that tralomethrin is itself intrinsically active. The lack of in vitro activity contrasts with the recommended field rates for this pyrethroid (0.013–0.0156 lb of a.i./acre), which are similar to those of deltamethrin [0.01 lb of a.i./acre (Hall, 1982)]. Tralomethrin is known to be unstable in ultraviolet light [Figure 3; see also Ruzo and Casida (1981)], in insect homogenates, and upon application to insects (Ruzo et al., 1981). We have found that in certain circumstances it debrominates even in insect saline (aqueous salt solution of neutral pH) in artificial light. The conclusion is that the activity of tralomethrin in the field is due to its debromination to deltamethrin. As such, tralomethrin represents the first propyrethroid to be given serious commercial consideration [cf. Briggs et al. (1983)].

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Registry No. Tralomethrin, 66841-25-6; deltamethrin, 52918-63-5.

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