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Utilization of an Infrared Detector for Selective Liquid Chromatographic Analysis. 2. Formulation Analysis of the Pyrethroid Insecticides Allethrin, Decamethrin, Cypermethrin, Phenothrin, and Tetramethrin

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The pyrethroid insecticides allethrin [2-methyl-4-oxo-3-(2-propenyl)-2-cyclopenten-1-yl 2,2-dimethyl-3-(2-methyl-1-propenyl)cyclopropanecarboxylate], decamethrin [(1*R*-[1α(*S**),3α])-cyano(3-phenoxyphenyl)methyl 3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropanecarboxylate], cypermethrin [cyano(3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate], phenothrin [(3-phenoxyphenyl)methyl 2,2-dimethyl-3-(2-methyl-1-propenyl)cyclopropanecarboxylate], and tetramethrin [phthalthrin; (1,3,4,5,6,7-hexahydro-1,3-dioxo-2*H*-isoindol-2-yl)methyl 2,2-dimethyl-3-(2-methyl-1-propenyl)cyclopropanecarboxylate] in formulated materials were analyzed with a high-performance liquid chromatographic system utilizing a 10-μm Partisil column at ambient temperature and an on-line infrared detector and a flow cell. Chromatograms demonstrating the selectivity, minimum detectability, and resolution of the various pyrethroid isomers achieved are presented. The mobile phase consisted of various combinations of CCl_4, $CHCl_3$, CH_2Cl_2, 1,2-dichloroethane, cyclohexane, heptane, and CH_3CN, depending on the pyrethroid under study.

The use of high-performance liquid chromatography (HPLC) for sample cleanup and analyte separation combined with an on-line infrared (IR) detector with a flow cell for selective analyte detection and quantitation is a potentially useful technique for insecticide formulation and residue analysis. Papadopoulou-Mourkidou et al. (1980) applied the technique to the formulation analysis of the pyrethroid insecticides resmethrin, permethrin, and fenvalerate. Data are presented here to demonstrate the general utility of the technique for the analysis of emulsifiable concentrate and aerosol formulations of the pyrethroid insecticides. Dilution of the concentrates was the only sample preparation required. Analyses were conducted on allethrin, cypermethrin, tetramethrin (phthalthrin), and phenothrin which are currently commercially available in the United States and on decamethrin which is available in western Europe.

Early in the developmental stages of the pyrethroid insecticides structure–activity correlations showed that proper stereochemistry is of primary importance for optimum insecticidal activity (Elliott and Janes, 1978). Pyrethroids generally have two to three chiral centers, and the appropriate chirality of these centers is of primary importance for optimum biological activity. In addition, chrysanthamate ester pyrethroids have cis/trans isomerism due to the cyclopropane ring. As stereospecific synthetic methods are generally not available, formulations usually contain mixtures of isomers. Analytical methods should provide for the possibility of separation and quantitation of the most biologically active isomer. The analytical systems reported herein can separate geometric isomers and diastereomers.

The method proposed by the Association of Official Analytical Chemists (AOAC, 1980) for technical-grade allethrin involves titration with sodium methoxide in pyridine of the chrysanthemic acid which is quantitatively liberated by the reaction of allethrin with ethylenediamine. *d-trans*-Allethrin is determined by gas chromatography (GC) utilizing a flame ionization detector (AOAC, 1980). The latter method is not applicable to formulations containing large amounts of the MGK Repellent 874 (2-hydroxyethyl *n*-octyl sulfide).

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Mestres et al. (1977) determined decamethrin residues in oranges field-treated with Decis by using GC–mass spectrometry.

Chapman and Harris (1979) used a GC with an electron-capture detector for the determination at the residue level (0.05 ppm in soil) of the C-1 (*R*/*S*) enantiomers of the cis/trans isomers of cypermethrin by making the *l*-menthyl esters of the acid moiety after hydrolysis of the pyrethroid ester. The same procedure was used to determine cypermethrin residues in vegetable crops after appropriate liquid–liquid partitioning and column chromatographic cleanup (Chapman and Harris, 1978). Nakayama et al. (1979) reported that *d-trans*-cypermethrin [1*R*-trans acid; *R*,*S* alcohol] can be separated on an open silica column eluted with 2.5% ethyl acetate in hexane into the two diastereomers. The 1*R*-trans,RC_α isomer eluted before the 1*R*-trans,SC_α isomer. The same order of elution of RC_α and SC_α isomers of NRDC 156 was reported by Meinard et al. (1979), who developed an HPLC–ultraviolet (UV) detector system for separation and quantitation of the most biologically active isomer 1*R*-cis,SC_α, decamethrin, in the presence of 1*R*-cis,RC_α and 1*R*-trans,*R*,SC_α on a Micropak column eluted with hexane–pentane–diethyl ether. Mourot et al. (1979) reported on the analysis of decamethrin in technical-grade material and in formulations using an HPLC–UV system operated in the normal phase with 7% diisopropyl ether in *n*-hexane and in reversed phase with 1% sulfuric acid–acetonitrile (30:70).

EXPERIMENTAL SECTION

d-trans-Allethrin (94.5%), D-Trans Aerosol Mix 1905, and D-Trans Intermediate 1862 were provided by McLaughlin Gormley King Co. Esbiol (97% *S*-bioallethrin) was provided by Sumitomo Chemical Co., Ltd., Japan.

Analytical (99.7%) and technical (98%) grade decamethrin (NRCD 161), NRDC 156, and Decis EC 2-C (0.21 lb of RU22974/U.S. gal) were provided by Procida-Roussel UCLAF, France.

Analytical-grade (98.1%) cypermethrin (NRDC 149) was provided by Shell Chemical Co., England. Analytical standards of *cis*- and *trans*-cypermethrin were provided by Dr. J. E. Casida of the University of California, Berkeley.

Analytical Standards (99%) of *cis*-permethrin (MRV 645) and *trans*-permethrin (MRV 449) and Pounce 3.2EC formulation (2 lb of *trans*-permethrin/gal) were provided by FMC Corp.

Technical-grade piperonyl butoxide was provided by Dr. T. A. Miller of the University of California, Riverside.

Sumithrin (93.8% *d*-phenothrin) was provided by Sumitomo Chemical Co., Ltd., Japan.

Technical-grade (88%) tetramethrin, Tetralate EC (formulation containing 2.5% tetramethrin and 2.5% resmethrin), and technical-grade MGK 264 synergist [*N*-(2-ethylhexyl)bicyclo[2.2.1]-5-heptene-2,3-dicarboximide] were provided by Fairfield American Corp.

Analytical-grade (99%) cycloprate (Zardex, hexadecyl cyclopropanecarboxylate) was provided by Zoecon Corp.

The HPLC system and detectors used were previously described by Papadopoulou-Mourkidou et al. (1980). All solvents were purchased from J. T. Baker Chemical Co. and were specifically sold for HPLC use. The various solvent mixtures used were vacuum-filtered through 0.5-μm filter paper and then degassed by shaking or sonification under reduced pressure. The final composition of the mobile phase was generally achieved by using a solvent programmer operated at isocratic conditions and a constant total flow rate of 1.2 mL/min unless otherwise noted. The injection volume was 10 μL by using a septumless injector (Papadopoulou-Mourkidou et al., 1980) unless it is noted that a Rheodyne 7125 loop injector (Cotati, CA) with a 20-μL loop was used with a loop-filling technique. All IR chromatograms were obtained at a detector setting of 0.1 AUFS and 1-mm slit width unless otherwise noted.

Quantitative measurements were made according to the external standard technique by peak height measurements. The carbonyl stretching vibration was utilized for all analyses due to its strong absorption in all pyrethroids. The 5.75-μm wavelength was found useful for the pyrethroids with the α-CN moiety and 5.80 μm for the others. The wavelengths selected also minimized overlapping interfering absorptions resulting from mobile-phase solvents.

RESULTS AND DISCUSSION

The use of an IR spectrophotometer equipped with a flow cell as a detection system for HPLC is still in the early developmental stages. The realization of the inherent disadvantages, vexing problems, and special precautions and requirements associated with the other HPLC detection systems currently being used has turned our attention to IR as a viable alternative detection system.

The IR detection system used here is a modest cost, easily operated, compact, filter type, tunable, variable-wavelength IR spectrophotometer. It shows good selectivity for formulation analysis, a wide linear range of response, and a great tolerance to changes in operating conditions such as flow rate and solvent gradient. It gives reliable and reproducible responses, is nondestructive to the analyte so that it can be isolated for further characterization or recycled for better purification, and shows promise for application to carbamate insecticides as well as to pyrethroids.

Although IR has certain requirements for the solvents used, other detectors also have similar solvent requirements. Whereas the previous report (Papadopoulou-Mourkidou et al., 1980) extensively used CCl_4, this generally undesirable solvent can either be minimized or entirely omitted by using mixtures of other solvents. Examples are given in the discussion below wherein several pyrethroids are separated in a mixture and where geometrical isomers and diastereomers of a pyrethroid are separated. These examples show the separations which are achievable in spite of the limited number of available IR-compatible solvents. Examples of a number of solvent systems are given to show what solvents can be successfully used.

The relatively high minimum detectable level is still a problem and will require the use of greater sample sizes, greater injection volumes, and use of other techniques for application of IR detection for residue analysis.

The discussion below will deal with the analysis of the pyrethroid insecticides allethrin, decamethrin, cypermethrin, phenothrin, and tetramethrin. Each pyrethroid is dealt with separately.

Allethrin. Allethrin, which is used as a rapid knockdown agent for indoor insect control, was the first commercialized pyrethroid. Allethrin has three chiral centers in addition to the cis/trans isomerism with respect to the cyclopropane ring and is thus a mixture of isomers. Allethrin formulations are usually made from technical-grade *d-trans*-allethrin (bioallethrin) which contains two diastereomers, one of which is *S*-bioallethrin [1(*R*),3(*R*)-*trans*,1(*S*)-allethrin], the most biologically active of the allethrin isomers.

Two formulations, an emulsifiable concentrate and an aerosol mixture, were analyzed. Both formulations contained the synergists MGK-264 and piperonyl butoxide in addition to *d-trans*-allethrin. The developed analytical

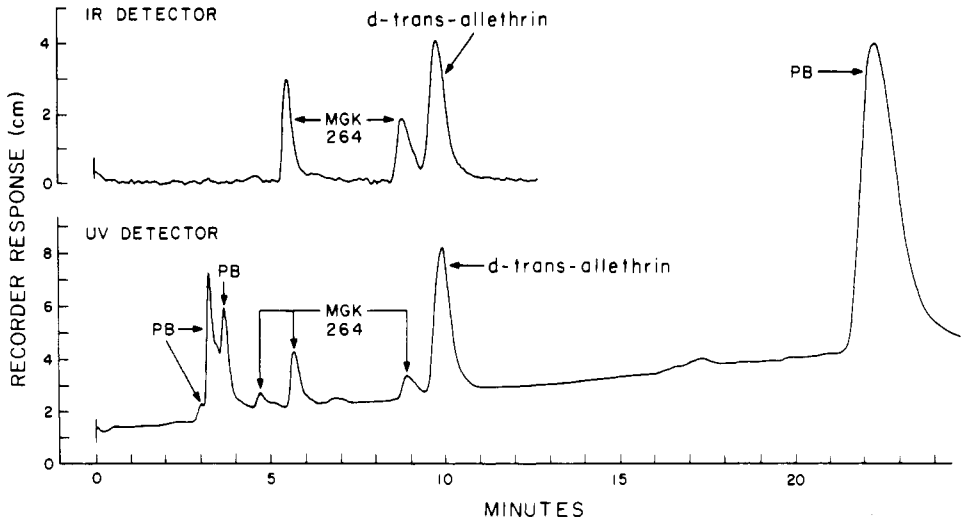

Figure 1. Chromatograms obtained after a 10-μL injection of a 4% (v/v) solution of D-Trans Intermediate 1862 in the mobile phase consisting of 3.8% (v/v) CH_3CN in CH_2Cl_2. The IR detector was operated at 5.80 μm; the UV detector was operated at 254 nm and 0.64 AUFS. PB denotes peaks arising from components in the piperonyl butoxide synergist.

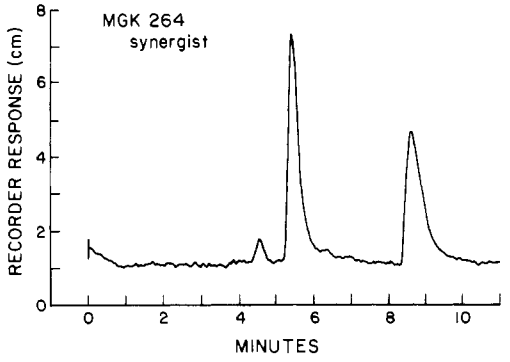

Figure 2. IR chromatogram obtained after a 10-μL injection of a 1% (v/v) solution of technical-grade synergist MGK 264 in the mobile phase consisting of 3.8% (v/v) CH_3CN in CH_2Cl_2. The detector was operated at 5.80 μm.

method for *d-trans*-allethrin also provides for the detection and quantitation of the synergist MGK-264.

Formulations were analyzed by using a mobile phase consisting of 3.8% (v/v) CH_3CN in CH_2Cl_2. The IR detector operated at 5.80 μm and 0.1 AUFS showed a linear response in the 5–50-μg range, a minimum detectable level of 5 μg, and a sensitivity of 0.11 cm/μg. At 0.025 AUFS the minimum detectable level was 2 μg. The on-line UV detector operated at 254 nm and 0.64 AUFS showed a linear response in the 5–50-μg range, a minimum detectable level of 5 μg, and sensitivity of 0.17 cm/μg. At 0.04 AUFS the minimum detectable level and sensitivity were 0.2 μg and 4 cm/μg at 254 nm and 10 μg and 0.1 cm/μg at 280 nm. Calibration curves were made by using the Esbiol (*S*-bioallethrin) standard. Standard solutions and formulation dilutions were made by using the mobile-phase solvent composition.

Figure 1 shows the chromatograms obtained for D-Trans Intermediate 1862 allethrin formulation. The IR chromatogram shows only three peaks, one due to *d-trans*-allethrin and two due to the technical-grade synergist MGK 264 which is also IR active at 5.80 μm due to two carbonyl groups. It is not known which of the two peaks is due to the active ingredient. Figure 2 shows the IR chromatogram for technical-grade MGK 264; the UV chromatogram was very similar in appearance. Piperonyl butoxide is not IR active at 5.80 μm; a 100-μg injection of the technical-grade material gave no response. The UV chromatogram of the allethrin formulation shown in Figure 1 shows numerous peaks due to the two synergists.

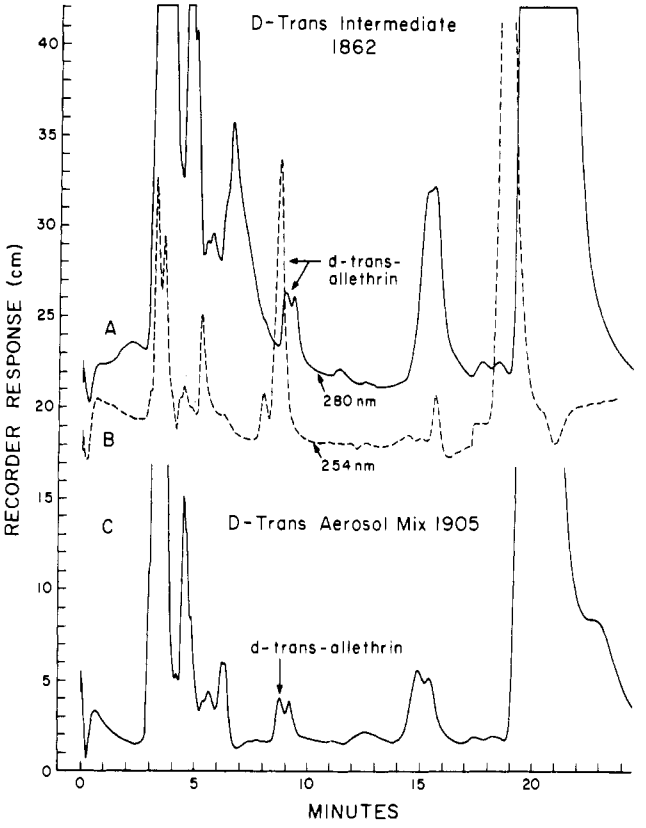

Figure 3. UV chromatograms obtained after separate 10-μL injections of the following three solutions prepared in the mobile phase consisting of 4% (v/v) CH_3CN in CH_2Cl_2: (A) 5% (v/v) and (B) 0.5% (v/v) D-Trans Intermediate 1862 and (C) 10% (v/v) D-Trans Aerosol Mix 1905. The detector was operated at 280 nm for (A) and (C) and at 254 nm for (B) and at 0.04 AUFS.

Figure 3 shows UV chromatograms of D-Trans Intermediate 1862 obtained at 254 and 280 nm and D-Trans Aerosol Mix 1905 obtained at 280 nm. The chromatograms of D-Trans Intermediate 1862 change dramatically in appearance, both qualitatively and quantitatively, with a change in the wavelength used. The chromatograms shown in Figure 1 and 3 demonstrate the superior selectivity of the IR detector for *d-trans*-allethrin and MGK 264. Since both these components are obscured in the UV chromatogram obtained at 280 nm, the 254-nm wavelength must

Table I. Results of the Analysis for *d-trans*-Allethrin in D-Trans Intermediate 1862 and D-Trans Aerosol Mix 1905 Allethrin Formulations Using an HPLC System with Tandem IR and UV Detectors

formulation	detector	range, AUFS	calibration factor, cm/μg	% (v/v) dilution	mean peak height, cm[a]	lb/gal found
D-Trans Intermediate 1862	IR, 5.8 μm	0.1	0.11	3	2.9	0.73
				4	3.7	0.70
	UV, 254 nm	0.64	0.17	3	4.3	0.69
				4	5.4	0.65
D-Trans Aerosol Mix 1905	IR, 5.8 μm	0.1	0.11	10	2.5	0.19
	UV, 254 nm	0.64	0.17	10	3.7	0.18

[a] Minimum of three injections.

be used. Analysis time for *d-trans*-allethrin is considerably longer by UV detection due to late-eluting UV-active components in the formulation.

Since a *cis*-allethrin standard was unavailable, the possibility was not excluded that *cis*-allethrin, if present, would elute at the same time as the MGK 264 peak just preceding the *trans*-allethrin peak. Since the ratio of 1.7 for the two MGK peaks obtained for the Technical-grade material (Figure 2) was also found for the chromatograms of the allethrin formulations, *cis*-allethrin is believed to be either not present or present in minute amounts below the detectable level.

The formulation analysis results are shown in Table I. The amount of *d-trans*-allethrin in D-Trans Intermediate 1862 was calculated to be 0.72 ± 0.02 lb/gal by using the IR detector and 0.67 ± 0.03 lb/gal by using the UV detector. The amount of *d-trans*-allethrin in D-Trans Aerosol Mix 1905 was calculated to be 0.19 lb/gal by using the IR detector and 0.18 lb/gal by using the UV detector.

Formulations can also be analyzed for *d-trans*-allethrin by using a mobile phase consisting of 4% CH_3CN, 16% CH_2Cl_2, and 80% cyclohexane (v/v/v). The IR chromatogram shows one peak for the Esbiol standard (*S*-bioallethrin) at a retention time of 7.8 min and one minor and two major peaks at retention times of 4.5, 5.4, and 9.0 min, respectively, for MGK 264. The minimum detectable level for this system is not as good as that for the system used above due to the use of cyclohexane in this system. Due to the high melting point (6.6 °C), pure cyclohexane should not be pumped when the system has a back pressure greater than about 2000 psi. Addition of 5% cyclopentane in cyclohexane allows safer use to the pumps without changing the elution characteristics of the mobile phase.

Decamethrin (NRDC 161). Decamethrin, one of the most biologically active pyrethroids, is one of the diastereomers of the stereospecifically synthesized pyrethroid NRDC 156 [(*R*,*S*)-cyano(3-phenoxyphenyl)methyl (1*R*,3*R*)-*cis*-3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropanecarboxylate]. Decamethrin is the (1*R*,3*R*)-cis,*SC*$_a$ diastereomer (diastereomer *S*). Decis EC 2-C is an emulsifiable concentrate formulation of decamethrin.

The IR spectrum of decamethrin in CCl_4 has five strong adsorption bands in the mid-IR region at 5.75, 6.25, 6.64, 8.95, and 9.25 μm. Under the HPLC conditions to be described, only two of these bands were useful for quantitative work. With a mobile phase consisting of a small percentage of CH_3CN in CCl_4, the 5.75- and 8.95-μm bands were quite useful; the sensitivity at 8.95 μm was about twice that at 5.75 μm. With a complex mobile phase consisting of a small percentage of CH_3CN in cyclohexane–CCl_4–heptane mixtures, only the 5.75-μm band was of practical utility due to solvent absorptions.

With a mobile phase of 1% CH_3CN in CCl_4 and a flow rate of 0.9 mL/min, the compound NRDC 156 showed two peaks on the IR chromatogram. The peak with retention time of 11.3 min was assigned as the diastereomer (*R*) and the other with retention time of 12.2 min as the (*S*) isomer. A 100-μg injection of technical-grade decamethrin showed a major peak corresponding to the diastereomer *S* and a preceding detectable, but not measurable, peak corresponding to the *R* isomer impurity, whereas 100 μg of analytical-grade decamethrin (NRDC 161) showed only one peak with a retention time of 12.2 min corresponding to the *S* isomer. Decis showed the same chromatographic profile as the technical-grade decamethrin but with an additional peak at 3 min, apparently due to an IR-absorbing impurity in the formulated material.

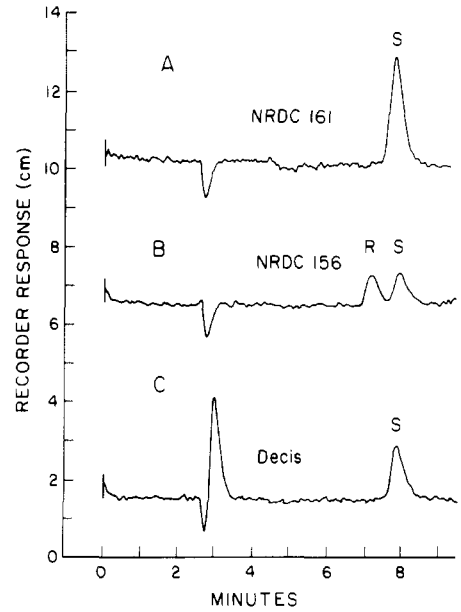

Figure 4. IR chromatograms obtained after 10-μL injections of (A) 100 μg of NRDC 161, technical decamethrin, in CCl_4, (B) 100 μg of NRDC 156 in CCl_4, and (C) a 20% (v/v) solution of Decis in CCl_4. The mobile phase was 70.0% cyclohexane, 28.5% CCl_4, and 1.5% CH_3CN (v/v/v). The detector was operated at 5.75 μm and with a 2-mm slit width.

The order of elution of the diastereomers, *R* preceding *S*, under the conditions reported herein was the same as that reported by Meinard et al. (1979), who also used normal-phase HPLC.

Figure 4 shows that by using a mobile phase consisting of 70% cyclohexane, 28.5% CCl_4, and 1.5% CH_3CN, it was possible to obtain better resolution of the two diastereomers as well as shortening the retention times to 7.2 and 8.0 min. The negative peak appearing at 2.8 min was due to the CCl_4 used to prepare the solutions.

Figure 5 shows the UV chromatograms for Decis and NRDC 156 using the same mobile phase as for Figure 4. The resolution of the diastereomer *R* from the major component, diastereomer *S*, is shown.

Under the conditions given in Figure 4, the IR detector showed for decamethrin a linear response between 10 and 100 μg, a minimum detectable level of 10 μg, and a sen-

Table II. Results of the Analysis of Decis Decamethrin Formulation Using an HPLC System with Tandem IR and UV Detectors

detector	range, AUFS	calibration factor, cm/μg	% (v/v) dilution	mean peak height, cm[a]	lb/gal found
IR,[b] 5.75 μm, slit 2 mm	0.1	0.03	10	0.7	0.20
		0.03	20	1.4	0.20
UV,[b] 280 nm	0.08	1.62	1	4.5	0.23
		1.62	2	8.75	0.23
IR,[c] 8.95 μm	0.1	0.093	10	2.3	0.21
		0.093	5	1.15	0.21
UV,[c] 280 nm	0.08	3.33	1	8.4	0.21

[a] Minimum of three injections. [b] Mobile phase consisting of 70% cyclohexane, 28.5% CCl_4, and 1.5% CH_3CN; 1.2 mL/min flow rate. [c] Mobile phase consisting of 5% CH_3CN in CCl_4; 0.9 mL/min flow rate.

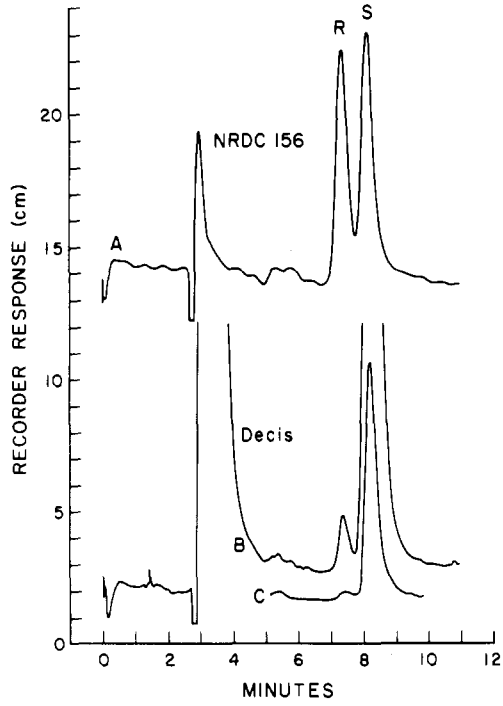

Figure 5. UV chromatograms obtained after 10-μL injections of (A) 10 μg of NRDC 156 in CCl_4 and (B) 10% (v/v) and (C) 2% (v/v) solutions of Decis in CCl_4. The mobile phase was 70.0% cyclohexane, 28.5% CCl_4, and 1.5% CH_3CN (v/v/v). The detector was operated at 280 nm and 0.08 AUFS.

sitivity of 0.03 cm/μg. Under the conditions given in Figure 5, the UV detector showed for decamethrin a linear response between 0.2 and 4.0 μg, a minimum detectable level of 0.2 μg, and a sensitivity of 1.6 cm/μg.

It is apparent from Figure 5 that diastereomer *R* is a minor component in the decamethrin formulation. The *R*/*S* ratio of the peak heights obtained by using the 2% Decis solution was 0.03. If comparable detector response for the two diastereomers is assumed, then the technical material used to prepare Decis formulation contained about 3% diastereomer R; Decis thus contained less than 1 g of diastereomer *R*/L. One might choose to analyze Decis for total pyrethroid and make no distinction in the diastereomers due to the low levels of diastereomer *R* present. By use of a mobile phase consisting of 5% (v/v) CH_3CN in CCl_4 at a 0.9 mL/min flow rate, both diastereomers eluted as a single peak at a retention time of 4.2 min. Analysis time was reduced by half. Under these conditions the IR detector could be operated at 8.95 μm. At 0.1 AUFS the IR detector showed a linear response in the range 2–60 μg, a minimum detectable level of 2.0 μg, and a sensitivity of 0.10 cm/μg. At 280 nm and 0.64 AUFS, the UV detector showed a linear response in the range 1–30 μg, a minimum detectable level of 1.0 μg, and a sensitivity of 0.4 cm/μg. At 0.08 AUFS the UV detector showed a

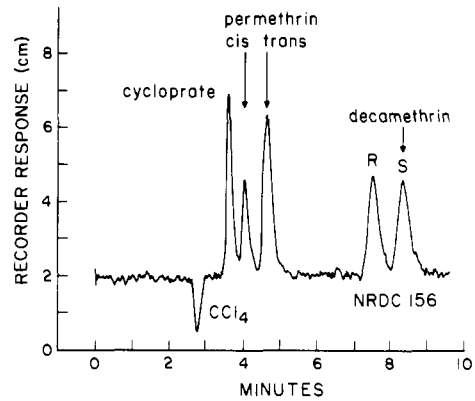

Figure 6. Sample IR chromatogram obtained after a 10-μL injection of a CCl_4 solution of 3% (v/v) Pounce 3.2EC permethrin formulation containing 5.0 mg/mL cycloprate and 20 mg/mL NRDC 156. The mobile phase was 94% cyclohexane, 4.8% CH_2Cl_2, and 1.2% CH_3CN (v/v/v). The detector was operated at 5.75 μm.

linear response in the range 0.1–1.0 μg, a minimum detectable level of 0.1 μg, and a sensitivity of 4.0 cm/μg.

Results of the analysis of Decis using the last two solvent systems mentioned above are given in Table II. By use of both the IR and UV detectors for quantitation, the cyclohexane–CCl_4–CH_3CN system gave a mean value of 0.22 ± 0.02 lb of decamethrin/gal and the CH_3CN–CCl_4 system gave a mean value of 0.21 lb of decamethrin/gal of Decis.

A mobile-phase system consisting of 94% cyclohexane, 4.8% CH_2Cl_2, and 1.2% CH_3CN (v/v/v) separated decamethrin from diasteromer *R* with retention times of 7.5 and 8.4 min, respectively. A sample chromatogram for the analysis of Pounce 3.2EC permethrin formulation fortified with cycloprate and NRDC 156 is given in Figure 6. If desired, cycloprate or either *cis*- or *trans*-permethrin isomers could be used as an internal standard for decamethrin formulation analysis.

Another IR-compatible mobile phase which could be used if diastereomer separation was necessary consisted of 59% cyclohexane, 35% heptane, 4.8% CH_2Cl_2, and 1.2% CH_3CN (v/v/v/v). With this mobile phase, NRDC 156 gave two peaks at 10.5 (*R*) and 11.8 (*S*) min; cycloprate and *cis*/*trans*-permethrin gave peaks at 4.0, 4.8 (cis), and 5.8 (trans) min, respectively; fenvalerate diastereomers gave peaks at 12.5 and 13.6 min.

Cypermethrin (NRDC 149). Cypermethrin is a mixture of eight isomers. A mobile phase of simply CH_2Cl_2 separates cypermethrin, technical material, into two peaks with retention times of 5.5 and 6.5 min. These peaks have been assigned to the cis and trans isomers of cypermethrin based on comparisons with individual analytical standards. With a mobile-phase system containing 53% cyclohexane, 45% CH_2Cl_2, 1.6% 1,2-dichloroethane, and 0.4% CH_3CN, the two cypermethrin isomers were separated with shorter

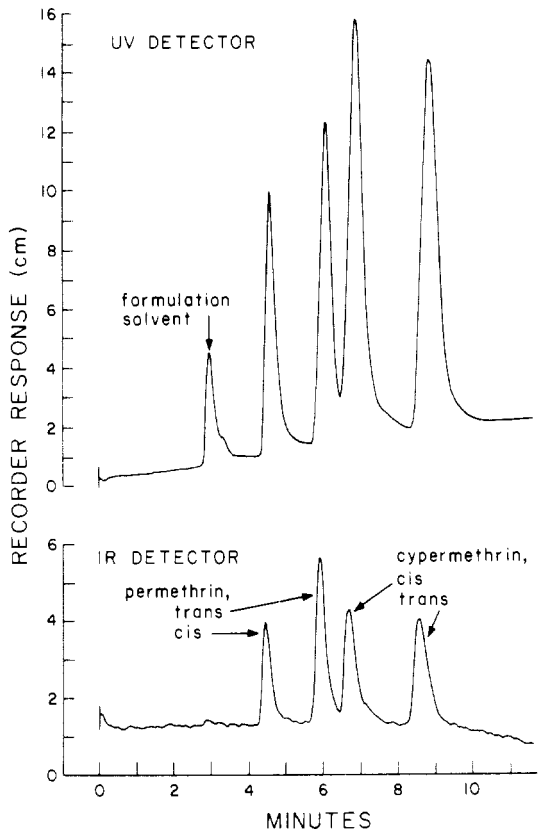

Figure 7. Chromatograms obtained after a 10-μL injection of a 3% (v/v) solution of Pounce 3.2EC permethrin formulation in the mobile phase consisting of 50% (v/v) CCl_4 in CH_2Cl_2; the solution also contained 7.0 mg/mL each of *cis*- and *trans*-cypermethrin. The IR detector was operated at 5.75 μm; the UV detector was operated at 280 nm and 0.64 AUFS.

retention times of 4.5 (cis) and 5.3 (trans) min; permethrin isomers were eluted at 3.3 (cis) and 4.0 (trans) min; cycloprate, a possible internal standard, was eluted at 4.9 min.

Since a cypermethrin formulation was unavailable, a simulated formulation was prepared by adding known amounts of *cis*/*trans*-cypermethrin or technical-grade cypermethrin to Pounce 3.2EC permethrin formulation. Figure 7 shows IR and UV chromatograms for the simulated formulation wherein *cis*- and *trans*-permethrin and *cis*- and *trans*-cypermethrin were separated from each other by using a mobile phase consisting of 50% CCl_4 in CH_2Cl_2.

Figure 8 shows chromatograms using a mobile phase consisting of 50% 1,2-dichloroethane in cyclohexane. Cypermethrin isomers eluted at 5.7 (cis) and 7.3 (trans) min; permethrin isomers eluted at 4.0 (cis) and 5.2 (trans) min; cycloprate eluted at 6.8 min. It is interesting to note that cycloprate, which eluted before both permethrin isomers under the conditions given in Figure 6, eluted after the permethrin isomers under the conditions given in Figure 8.

Cypermethrin was resolved into four peaks with retention times of 9.9, 11, 11.5, and 12.8 min by using a mobile phase consisting of 35% heptane, 59% cyclohexane, 1.2% CH_3CN, and 4.8% CH_2Cl_2. Cycloprate eluted at 4 min. It has previously been mentioned that this system also separates the NRDC 156 and fenvalerate diastereomers.

Evaluation of the HPLC system with an IR detector for cypermethrin formulation analysis was made by using only the 50% CCl_4 in CH_2Cl_2 system due to the lack of adequate amounts of standards. With the IR detector operated at 5.75 μm and 0.1 AUFS, the linear response was in the range

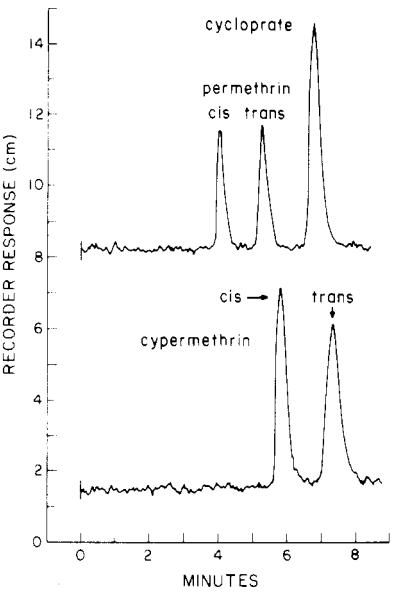

Figure 8. Sample IR chromatograms obtained after 20-μL loop-filling injections of (top) a solution containing 2.5 mg/mL each of *cis*- and *trans*-permethrin and 5.0 mg/mL cycloprate and (bottom) 300 μg of cypermethrin. The mobile phase was 50% 1,2-dichloroethane in cyclohexane. The detector was operated at 5.75 μm.

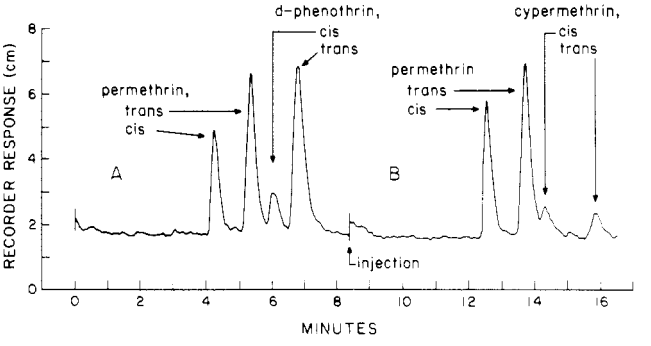

Figure 9. IR chromatograms obtained after 10-μL injections of a 3% (v/v) solution of Pounce 3.2EC permethrin formulation in the mobile phase consisting of 50% (v/v) CCl_4 in CH_2Cl_2 and containing (A) 94 μg of *d*-phenothrin and (B) 30 μg each of *cis*- and *trans*-cypermethrin. The detector was operated at 5.80 μm.

5–80 μg for cypermethrin and 3–80 μg for permethrin; the minimum detectable levels were 5 μg for cypermethrin and 3 μg for permethrin; the sensitivities were 0.04 cm/μg for cypermethrin and 0.06 cm/μg for permethrin; both geometrical isomers behaved identically.

Phenothrin. When a mobile phase consisting of 50% CCl_4 in CH_2Cl_2 was used, technical-grade *d*-phenothrin (Sumithrin) separated into two peaks at retention times of 6.1 and 6.9 min. Since individual analytical standards of the two geometrical isomers were unavailable, the identity of the two peaks could not be directly assigned. The early-eluting peak was assigned the cis configuration based on the general finding with other pyrethroids that the cis isomer elutes first under normal-phase HPLC conditions.

As a phenothrin formulation was unavailable, a simulated formulation was prepared by adding technical-grade *d*-phenothrin to Pounce 3.2EC permethrin formulation. Figure 9 shows the IR chromatogram of this simulated formulation using the conditions specified in the figure caption. Figure 9 also shows the chromatogram obtained for a simulated cypermethrin formulation under the same HPLC conditions. The wavelength used was 5.80 μm whereas cypermethrin analysis in the previous section used

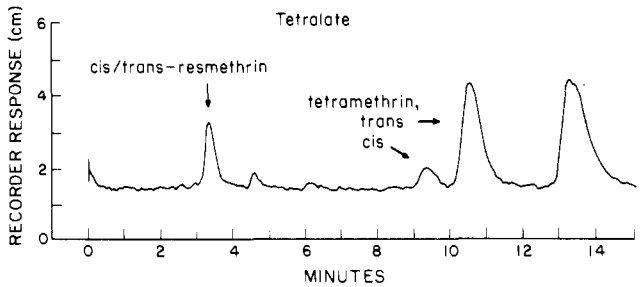

Figure 10. IR chromatogram obtained after a 10-μL injection of a 10% (v/v) Tetralate solution in CCl_4. The detector was operated at 5.80 μm. The mobile phase was 42.5% CCl_4, 42.5% CH_2Cl_2, 14.85% $CHCl_3$, and 0.15% CH_3CN (v/v/v/v).

5.75 μm. Comparison of Figures 7 and 9 indicates the selectivity of the IR detector where response to one compound over another can be enhanced simply by careful selection of the wavelength used.

No data are presented for the UV detector as the mobile phase apparently underwent photodecomposition and gave erratic responses.

When a mobile phase consisting of 50% 1,2-dichloroethane in cyclohexane was used, the two permethrin isomers eluted at 4.0 (cis) and 5.1 (trans) min; the *d*-phenothrin isomers eluted at 5.7 and 6.4 min. Resmethrin also separated into the cis (6.4 min) and trans (7.2 min) isomers.

Tetramethrin. Tetramethrin (phthalthrin) is a good knockdown agent and is usually formulated with other pyrethroids which have greater killing activity. Tetralate is a formulation which contains tetramethrin and resmethrin.

Figure 10 shows the IR chromatogram obtained for Tetralate by using a mobile phase of 42.5% CCl_4, 42.5% CH_2Cl_2, 14.85% $CHCl_3$, and 0.15% CH_3CN. The geometrical resmethrin isomers were unresolved and eluted as a single peak. The HPLC conditions for resmethrin formulation analysis were given by Papadopoulou-Mourkidou et al. (1980). The *cis*- and *trans*-tetramethrin isomers were resolved and eluted at retention times of 9.4 and 10.5 min. It was assumed that the cis isomer eluted before the trans isomer as individual analytical standards were unavailable for confirmation. Two peaks were not identified and are believed to arise from the formulating ingredients.

LITERATURE CITED

AOAC "Official Methods of Analysis of the AOAC", 13th ed.; Horwitz, W., Ed.; AOAC: Washington, DC, 1980; pp 86-87.
Chapman, R. A.; Harris, C. R. *J. Chromatogr.* **1978**, *166*, 513.
Chapman, R. A.; Harris, C. R. *J. Chromatogr.* **1979**, *174*, 369.
Elliott, M.; Janes, N. F. *Chem. Soc. Rev.* **1978**, *7*, 473.
Meinard, C.; Suglia, J. C.; Bruneau, P. *J. Chromatogr.* **1979**, *176*, 140.
Mestres, R.; Chevallier, C.; Espinoza, C.; Cornet, R. *Proc. Int. Soc. Citric., 1977* **1977**, *2*, 429.
Mourot, D.; Delepine, B.; Boisseau, J.; Goyot, G. *J. Chromatogr.* **1979**, *173*, 412.
Nakayama, I.; Ohno, N.; Aketa, K. Suzuki, Y.; Kato, T.; Yoshioka, H. In "Advances in Pesticide Science"; Geissbuehler, H., Ed.; Pergamon Press: New York, 1979; Vol. II, pp 174-181.
Papadopoulou-Mourkidou, E.; Iwata, Y.; Gunther, F. A. *J. Agric. Food Chem.* **1980**, *28*, 1043.

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Insecticidal Phosphoramidothio Derivatives of the Carbamate Methomyl

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The insecticidal activities of a number of phosphoramidothio derivatives of methomyl were investigated. The derivatives demonstrated activity comparable to that of methomyl in feeding tests against southern armyworm (*Spodoptera eridania*, Cramer), cabbage looper (*Trichoplusia ni*, Hubner), and tobacco budworm (*Heliothis virescens*, Fabricius). The topical activities were generally equal or superior to methomyl while ovicidal activities were less than those of methomyl against the same species. The derivatives demonstrated longer residual activity and reduced phytotoxicity relative to methomyl. Toxicological evaluation of one of the compounds (methyl *N*-[[[[[(diethoxyphosphinothioyl)isopropylamino]thio]methylamino]carbonyl]oxy]ethanimidothioate, U-47319) showed a substantial improvement in acute mammalian safety toward male and female rats when compared to that of methomyl.

The modification of carbamate insecticides by substitution of the carbamate nitrogen with moieties which alter the ancillary properties of the parent compound, particularly toxicity toward nontarget organisms, has been the object of intensive research in both academia and industry. Noteworthy among the substitutive groups employed for this purpose are *N*-thio derivatives. Arylthio (Black et al., 1973a), aminothio (Fukuto et al., 1975), and *N*-carbamylthio (Fahmy et al., 1978) derivatives of a number of carbamate insecticides show reduced mammalian toxicity. Aminothio derivatives of methomyl are reported to have reduced phytotoxicity and longer residual effectiveness as well as reduced mammalian toxicity (Gemrich et al., 1978). The increased selectivity of *N*-thio carbamates has been attributed to differential metabolism (Black et al., 1973b; Krieger et al., 1976). We report here the results of our investigations of phosphoramidothio derivatives of methomyl.

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EXPERIMENTAL SECTION

Synthesis of Compounds. The phosphoramidothio derivatives (Table I) were obtained by the reaction of an *N*-chlorothiophosphoramide (NCP) with methomyl in the presence of a catalytic amount of cuprous chloride (Figure 1). The chlorothiophosphoramides were prepared by the reaction of sulfur dichloride with the phosphoramides

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