imidothioate (V, U-46,855) is a potentially useful insect control agent and that modification of a good insecticidal chemical can result in the production of compounds with improved toxicological and biological parameters.

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Determination of FMC 33297 Residues in Plant, Animal, and Soil Matrices by Gas Chromatography

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Analytical procedures are described for the determination of FMC 33297 residues in/on various food and fiber crops, soils, and animal tissues and fats. The quantitative methods involve extraction of residues from the sample matrices using hexane, hexane–isopropanol, or methanol–water, depending on sample type followed by cleanup on Florisil. Samples containing oils and lipids are eluted through a gel permeation column prior to Florisil column cleanup. Residues of FMC 33297 are detected by gas–liquid chromatography utilizing either a ^{63}Ni electron-capture detector of a Coulson electrolytic conductivity detector operating in the halogen mode. Adequate recoveries are obtained from fortified check samples spiked at the 0.05 ppm level with each isomer of FMC 33297 in all sample types analyzed.

FMC 33297, 3-phenoxybenzyl (±)-*cis,trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate, is a synthetic pyrethroid insecticide currently being developed by FMC Corporation. FMC 33297 (Figure 1), also known as NRDC 143 and permethrin, has been described by Elliot et al. (1973). FMC 33297 has a cis,trans isomer ratio of: minimum 35% (±)-cis and maximum 65% (±)-trans.

As part of the development process, analytical procedures were required for the determination of FMC 33297 residues in/on various food and fiber crops. Williams (1976) reported an analytical procedure for the analysis of permethrin in potato tubers without separation of the *cis,trans*-permethrin isomers. This paper describes analytical procedures developed by FMC Corporation for the analysis of FMC 33297 residues with isomer separation in/on various food and fiber crops, soils, and animal fats and tissues.

The analytical procedures involve extraction of residues from the sample matrices using organic solvents followed by cleanup on activated Florisil. Samples containing high oil or lipid content are subjected to gel permeation cleanup prior to elution through Florisil. The use of gel permeation chromatography for pesticide–lipid separation was reported by Stalling et al. (1972, 1974). Tindle and Stalling (1972) described an automated gel permeation system capable of processing 23 samples unattended. Griffitt and Craun (1974) evaluated the automated system and found it to be an efficient tool for fat–pesticide separation.

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EXPERIMENTAL SECTION

Reagents. All reagents used were purchased commercially as pesticide quality or equivalent from Burdick and Jackson Laboratories, Inc., and from Mallinckrodt Chemical Co. Florisil, PR Grade, 60–100 mesh, Floridin Co., was activated overnight at 135 °C prior to use.

Apparatus. An automated gel permeation system, GPC Autoprep 1001, Analytical Biochemistry Laboratories, Inc., was used. The columm, 2.5 cm × 30 cm, was packed with 50 g of Bio-Beads S-X3, 200–400 mesh, Bio-Rad Laboratories, compressed to a bed length of 27 cm using Kontes Organic Solvent plunger assemblies (K-422353-0025). A 1:3 mixture of hexane–ethyl acetate was used as the eluting solvent with a flow rate of 5 mL/min. Operating time parameters for the dump, collect, and wash cycles were 17, 8, and 4 min, respectively.

A Tracor MT-220 gas chromatograph equipped with both a ^{63}Ni electron-capture detector (ECD) and a Coulson electrolytic conductivity detector (CCD) operating in the halogen mode was used. A noise filter, Spectrum Model 1021, was placed in-line between the ECD and the strip chart recorder to increase baseline stability. The ECD was connected to a 6 ft × 2 mm i.d. glass column packed with Supelcoport, 80/100 mesh, coated with 1% SP-2330. The

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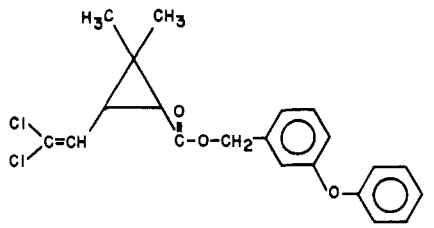

FMC 33297

Figure 1.

glass column was silane treated according to the procedure of Leibrand and Dunham (1973) using hexamethyldisilazane instead of dimethydichlorosilane prior to packing. The inlet block, column oven, transfer block, and detector temperatures were maintained at 275, 215, 275, and 325 °C, respectively. Nitrogen carrier gas flow rate was 20 mL/min and detector purge gas flow was 60 mL/min. The ECD was operated in the DC mode. The CCD was connected to a 6 ft × 2 mm i.d. glass column packed with Supelcoport, 80–100 mesh, coated with 5% SP-2330. The glass column was also silane treated prior to packing. The inlet block, column oven, and transfer block temperatures were maintained at 275, 215, and 275 °C, respectively. The CCD value and furnace temperatures were maintained at 280 and 850 °C, respectively. Hydrogen carrier gas flow rate was 100 mL/min and hydrogen reactor gas flow rate was 20 mL/min. The CCD bridge circuit was modified to increase the detector cell DC voltage from 30 to 60 V according to the procedure of Dolan and Hall (1973). The CCD sensitivity was also increased by reducing the rate of water flow through the cell system using a suitable length of stainless steel wire (0.003 in diameter) according to the procedure of Lawrence and Sen (1975).

Extraction: Low-Moisture Crops. Twenty-gram subsamples of macerated crop, e.g., soybeans, ginned cottonseed, flue-cured tobacco, were blended for 5 min in 100 mL of hexane. Approximately 40 g of anhydrous sodium sulfate was added, and the samples were blended for an additional 2 min. Celite was added, and the samples were filtered through Buchner funnels lined with glass fiber filter paper. The filter cakes and papers were returned to the blenders and blended for 3 min using a second 100-mL portion of hexane. The samples were filtered and the blending jars and filter cakes rinsed with 50 mL of blending solvent. The volume of each sample was adjusted to 250 mL with hexane.

Extraction: High-Moisture Crops. Twenty-grams subsamples of macerated crop, e.g., lettuce, cole crops, tomatoes, were extracted as described previously using a 2:1 mixture of hexane–isopropanol. Sodium sulfate was not used in this procedure. The combined filtrates from the blending procedure were transferred to 500-mL separatory funnels, and 100 mL of distilled water and 10 g of sodium chloride were added to each sample. The samples were shaken for 1 min and the phases allowed to separate. The lower aqueous phase of each sample was transferred to a second separatory funnel and extracted with 50 mL of hexane. The aqueous phases were discarded and the hexane fractions filtered through anhydrous sodium sulfate into graduated cylinders. The volume of each sample was adjusted to 250 mL with hexane.

Extraction: Soil. Fifty-gram subsamples of air-dried and screened soil were blended and filtered as described previously using a 9:1 mixture of methanol–water. The volume of the methanol–water mixtures were adjusted to 250 mL and 50-mL aliquots were transferred to 250-mL separatory funnels. The samples were diluted with 50 mL of distilled water and 10 g of sodium chloride was added. Samples were extracted 3 × 50 mL with methylene chloride. Each portion of methylene chloride was filtered through anhydrous sodium sulfate and all three portions collected in 250-mL Phillips beakers and held for analysis.

Concentration. Aliquots (2–10 g) of sample extracts were transferred to 125-mL Phillips beakers and concentrated on a steam table under a gentle stream of nitrogen. Hexane extracted samples were concentrated to a final volume of 2–3 mL in hexane. High-moisture crop extracts and soil extracts were concentrated to a final volume of 2–3 mL in toluene to insure evaporation of any remaining polar extraction solvents. Samples not requiring oil or lipid removal were eluted directly through Florisil and analyzed. Samples requiring gel permeation cleanup prior to Florisil cleanup were quantitatively transferred to 12-mL graduated centrifuge tubes using hexane as the rinse solvent and concentrated to 1–2 mL in warm water bath under a stream of nitrogen. The concentrated samples were diluted to 10.0 mL using a 1:3 mixture of hexane–ethyl acetate and centrifuged at 2500 rpm for 3 min to settle out any colloidal material present.

Gel Permeation Cleanup. The samples were injected into the 5.0-mL capacity sample loops of the GPC Autoprep 1001. Approximately 8 mL of sample was injected with the excess sample being discarded by the sample introduction valve system. The appropriate parameters were set and the samples sequentially eluted through the gel column. The 85–125-mL fractions of column eluates were collected in 125-mL Phillips beakers and concentrated to 2–3 mL in toluene using the evaporation procedure previously described.

Florisil Cleanup. Glass chromatographic columns, 1.5 cm o.d. × 17 cm, were packed with 4 g of activated Florisil and capped with a 1.5-cm layer of anhydrous sodium sulfate. The columns were prewashed with 20 mL of a 9:1 mixture of hexane–anhydrous diethyl ether followed by 2 × 5 mL hexane rinses. The samples were transferred to the columns using 2 × 2 mL hexane rinses. Elution of the columns was carried out using 40–55 mL of a 9:1 mixture of hexane–diethyl ether. The exact volume of eluting solvent used was dependent upon the retention characteristics of the batch of Florisil used. The column eluates were concentrated into a suitable volume of hexane or toluene for gas chromatographic analysis.

Gas Chromatographic Analysis. Quantitation of residues was accomplished by measurement of peak heights. Detector response calibration curves, peak height vs. amount of chemical injected, were plotted daily using a standard solution of FMC 33297. Separate calibration curves were plotted for the cis and trans isomers of FMC 33297. For the CCD, a typical calibration curve ranged from 2–10 ng at attenuation IX. For the ECD, a typical calibration curve ranged from 20–80 pg at attenuation 4 × 10^2.

RESULTS AND DISCUSSION

Gel permeation chromatography proved to be an efficient tool for pesticide–lipid separation in FMC 33297 residue analysis. Excellent pesticide–lipid separation was achieved by using Bio-Beads S-X3 and the hexane–ethyl acetate solvent system.

In the gas chromatographic analysis of FMC 33297, liquid phases DEGS-PS, OV-210, OV-225, and SP-2330 provided separation of the cis,trans FMC 33297 isomers when using the ECD. For the CCD, DEGS-PS, and SP-2330 provided adequate isomer separation, but OV-210

Table I. Method Recovery of FMC 33297 from Plant, Animal, and Soil Samples

Sample type	No. of analyses	Fortification,[a] ppm	Av % recov[d]	
			Cis	Trans
Lettuce	16	0.05-2.0	86 (70-100)	85 (70-105)
Cabbage	11	0.05-0.20	81 (68-92)	87 (75-94)
Cauliflower	4	0.05-0.10	95 (86-102)	90 (80-106)
Broccoli	4	0.05-0.50	85 (70-91)	84 (68-93)
Brussel Sprouts	5	0.05-0.20	83 (80-86)	89 (83-98)
Soils	12	0.05-0.50	92 (82-100)	91 (80-97)
Flue-cured tobacco	4	0.05-4.0	95 (86-102)	94 (92-100)
Ginned cottonseed	23	0.05	89 (84-100)	92 (72-100)
Soybeans	6	0.05	94 (84-104)	94 (76-106)
Tomatoes	11	0.02-0.50	89 (75-104)	90 (70-110)
Refined oil[b]	4	0.05	73 (66-92)	75 (64-80)
Solvent-extracted meal[b]	5	0.05	88 (76-96)	86 (70-92)
Alkaline soap stock[b]	5	0.05-0.10	83 (66-95)	84 (66-104)
Total fatty acids[b]	4	0.05	90 (77-112)	93 (83-110)
Tomato juice[c]	2	0.02-0.05	72 (68-75)	80 (70-90)
Tomato puree[c]	2	0.02-0.05	75 (70-80)	71 (70-72)
Tomato pomace[c]	2	0.05	82 (80-84)	80
Rat renal fat	4	0.05-5.0	73 (68-76)	77 (66-80)
Rat liver	3	0.05	88 (84-92)	88 (80-100)
Mice peritoneal fat	8	0.1-10	74 (68-80)	78 (68-83)

[a] Each isomer of FMC 33297. [b] Soybean and cottonseed processing products. [c] Tomato processing products. [d] Recovery range in parenthesis.

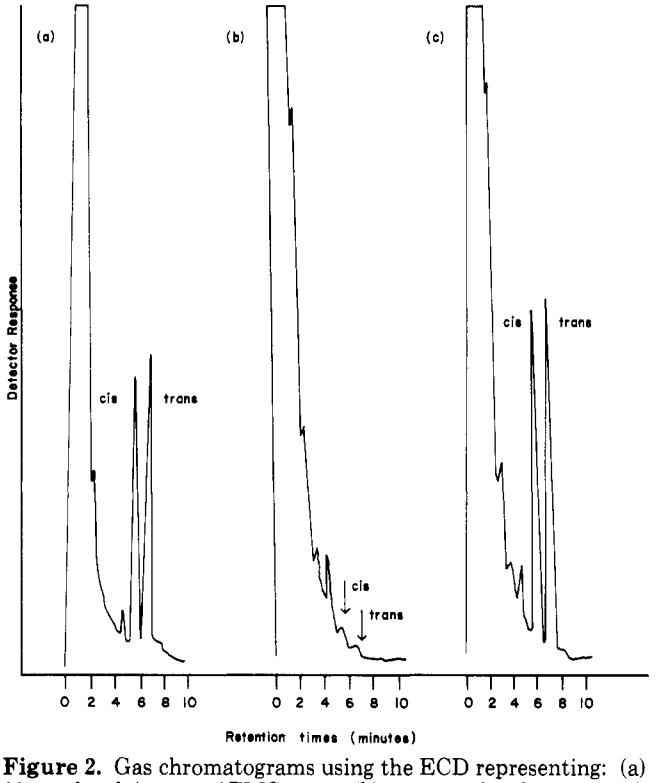

Figure 2. Gas chromatograms using the ECD representing: (a) 30 pg of each isomer of FMC 33297, (b) 0.75 mg of soybean control, (c) 0.75 mg of soybean control fortified at 0.05 ppm each isomer of FMC 33297.

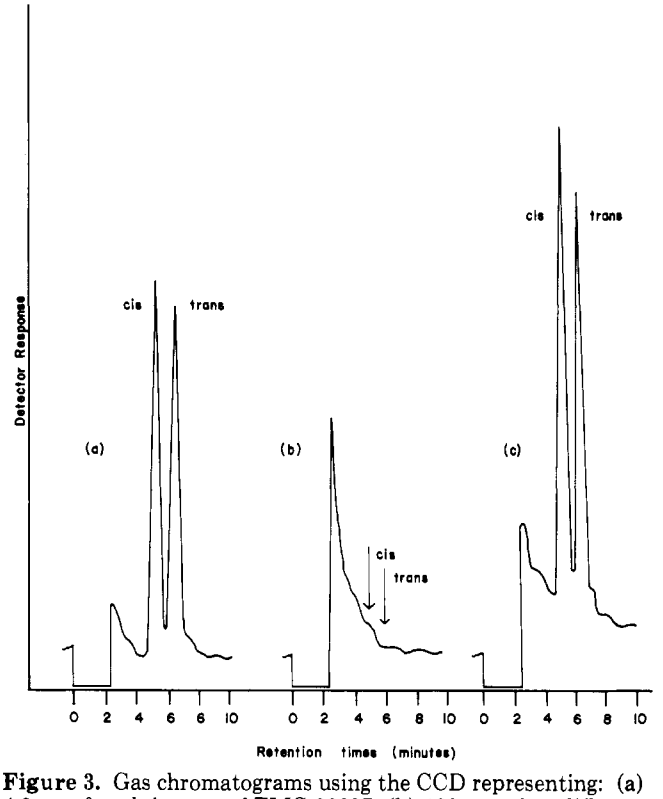

Figure 3. Gas chromatograms using the CCD representing: (a) 4.0 ng of each isomer of FMC 33297, (b) 100 mg of cauliflower control, (c) 100 mg of cauliflower control fortified at 0.05 ppm each isomer FMC 33297.

and OV-225 did not. SP-2330 was chosen for use with both detectors because of its higher thermal stability than DEGS-PS. For all liquid phases, the order of elution was cis isomer followed by trans. Higher stationary phase loading was required for columns used with the CCD than the ECD to obtain adequate FMC 33297 isomer separation.

Recovery data for crop and animal samples fortified with FMC 33297 is tabulated in Table I. Recovery was adequate for both isomers of FMC 33297 in all sample types analyzed at the 0.05 ppm fortification level. No attempts were made to determine the minimum method sensitivity of the described procedure for the various sample types analyzed. In Figure 2 GLC chromatograms are shown for the analysis of soybean samples using the ECD. In Figure 3 chromatograms are shown for the analysis of cauliflower samples using the CCD.

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Crystal and Molecular Structure of Organophosphorus Insecticides. 7. Crufomate

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The crystal and molecular structure of crufomate {2-chloro-4-(1,1-dimethylethyl) methyl methylphosphoramidate, $(H_3CO)(H_3CNH)P(O)OC_6H_3ClC(CH_3)_3$, triclinic, $P\bar{1}$, a = 9.506 (2), b = 11.826 (3), c = 7.277 (1) Å, α = 106.90 (2), β = 91.86 (3), γ = 105.11(3)°, Z = 2, Mo Kα radiation} has been determined by three-dimensional x-ray analysis. The structure was solved by conventional Patterson and Fourier techniques to a final discrepancy index R = 0.074 for 1632 observed reflections ($F_0 > 2.5\sigma(F_0)$). The configuration is substantiated by CNDO II molecular orbital calculations and van der Waals arguments and likely features a weak electrostatic interaction which somewhat restricts rotation about the phenolic C–O bond, thus giving a probable in vivo model. The phosphorus–meta hydrogen distances of 5.68 and 5.13 Å fall well within the range of literature values cited for the intramolecular active site–separation distance for insect acetylcholinesterase (AchE), yet are well outside that for mammalian AChE.

The crystal structure investigation of crufomate was undertaken as a part of a study of various organophosphorus (OP) insecticides being carried on at this laboratory (Baughman and Jacobson, 1975, 1976, 1977; Gifkins and Jacobson, 1976; Rohrbaugh and Jacobson, 1976, 1977). The purpose of such a program is to better understand the relationship between structure and mechanism(s) relative to an insecticide's toxicity/activity.

In the case of acetylcholinesterase (AChE) inhibition, one would ideally like to know the three-dimensional structure of the active sites (or that of a small range of possible low-energy conformations). The complexity of this enzyme virtually prohibits direct elucidation of its structure. However, accurate structural determinations of smaller molecules such as the organophosphorus (OP) and/or carbamate insecticides, which strongly interact with the active site(s) of AChE, would allow not only inferences to be made with regards to the topography of AChE but also yield valuable molecular insight into the insecticides themselves. Such information could then lead to the construction of insecticides which would better conform structurally as well as chemically to the most favorable (minimum energy) orientation of AChE.

EXPERIMENTAL SECTION

Preparation. A sample of 99% pure crufomate was recrystallized from reagent grade carbon tetrachloride. It was necessary to evaporate the solution to dryness to obtain the colorless species.

Crystal Data. A rectangular prismatic crystal with approximate dimensions 0.30 mm × 0.40 mm × 0.50 mm was selected and mounted on the end of a glass fiber using Elmer's Glue-All. The crystal was then mounted on a four-circle diffractometer; three ω-oscillation photographs taken at various χ and ϕ settings indicated that the crystal was indeed single.

From these photographs 16 independent reflections were selected and their coordinates were input into an automatic indexing program (Jacobson, 1976). The reduced cell and reduced cell scalars which resulted indicated triclinic symmetry, which was confirmed by inspection of ω-oscillation photographs taken about each of the three axes in turn. No axis showed mirror symmetry. Observed layer line spacings agreed, within experimental error, with those predicted for this cell by the indexing program.

The lattice constants were obtained from a least-squares refinement based on the precise $\pm 2\theta(|2\theta| > 30°)$ measurements of 22 strong independent reflections. At 27 °C using Mo Kα (λ = 0.70954 Å) they are a = 9.506 (2), b = 11.826 (3), c = 7.277 (1) Å, α = 106.90 (2), β = 91.86 (3), γ = 105.11 (3)°. The observed density of 1.30 ± 0.03 g cm^{-3} determined by the flotation method is in good agreement with the calculated value of 1.292 g cm^{-3} for two molecules with a molecular weight of 292.1 g mol^{-1} in a unit cell having a volume of 750.49 Å^3.

Collection and Reduction of X-Ray Intensity Data. The data were collected at 27 °C with graphite monochromated Mo Kα radiation on an automated four-circle diffractometer designed and built in the Ames Laboratory and previously described by Rohrbaugh and Jacobson (1974). All data within a 2θ sphere of 45° {$(\sin\theta)/\lambda$ = 0.539 Å^{-1}} in the hkl, $\bar{h}kl$, $h\bar{k}l$, and $\bar{h}\bar{k}l$ octants were measured using an ω–stepscan technique.

As a general check on electronic and crystal stability, the intensities of three standard reflections were remeasured every 75 reflections. These standard reflections were not observed to vary significantly throughout the entire period of data collection (~2 days). Hence, a decomposition correction was unnecessary. A total of 2993 reflections were recorded in this manner. Examination of the data did not reveal any systematic extinctions. As a Howells, Phillips, and Rogers (1950) plot indicated a centrosymmetric space group, the space group was assigned as $P\bar{1}$.

The intensity data were corrected for Lorentz and polarization effects and, since μ = 3.64 cm^{-1}, absorption corrections were not made; maximum and minimum transmission factors were 0.897 and 0.834, respectively. The estimated variance in each intensity was calculated by the following equation:

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