the different varieties, the flavor quality unfortunately not being in favor of the most commercially cultivated varieties.

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Determination of Chlorpyrifos and 3,5,6-Trichloro-2-pyridinol Residues in Peppermint Hay and Peppermint Oil

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Procedures are described for the determination of the insecticide *O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate (chlorpyrifos) and its metabolite 3,5,6-trichloro-2-pyridinol in mint hay and oil. After extraction, the chlorpyrifos residues are cleaned up on a silica gel column and quantitated by phosphorus-specific gas chromatography. The metabolite is separted from the extraction solvent by liquid–liquid partitioning with aqueous sodium carbonate, followed by chromatography on acid alumina. The column eluate is treated with *N,O*-bis(trimethylsilyl)acetamide and analyzed by electron capture gas chromatography. These methods are sensitive to 0.02 ppm in hay and to 0.1 ppm for the parent compound and 0.5 ppm for the metabolite in oil. Recoveries averaged 88%.

Field trials with Lorsban insecticide containing chlorpyrifos [*O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate] as the active ingredient were encouraging for the control of red-backed cutworm and mint root borer in mint (Berry, 1978, 1979). As a result analytical methods for the analysis of this compound and its principal metabolite (3,5,6-trichloro-2-pyridinol) in mint hay and oil were needed. While a number of procedures for the analysis of chlorpyrifos in agricultural products have been described (Bowman and Beroza, 1967; Braun, 1974; McKellar, 1972, 1973; Maini and Collina, 1972), none were directly applicable to mint hay and oil and none provided for analysis of both chlorpyrifos and the pyridinol metabolite in a single sample extract. Struble and MacDonald (1973) and Dishburger et al. (1977) described column chromatographic procedures providing cleanup for chlorpyrifos in wheat and for the pyridinol metabolite in bovine tissues, respectively. This paper reports modifications of these methods to allow separation of sample extracts into chlorpyrifos and 3,5,6-trichloro-2-pyridinol fractions and to provide adequate removal of coextracted plant material.

EXPERIMENTAL SECTION

Apparatus and Reagents. A Varian 3740 gas chromatograph equipped with flame photometric and electron capture detectors was used. Standard laboratory glassware was used throughout the procedure. Analytical-grade chlorpyrifos [*O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate] and 3,5,6-trichloro-2-pyridinol were obtained from Dow Chemical Co., Midland, MI. *N,O*-Bis(trimethylsilyl)acetamide (BSA) was obtained from Pierce Chemical Co., Rockford, IL. All solvents were distilled in glass.

Gas Chromatography. A flame photometric detector operated with the 530-nm interference filter for phosphorus was used for the determination of chlorpyrifos. The detector temperature was 230 °C with gas flows to the detector of 140, 80, and 170 mL/min for hydrogen, air 1, and air 2, respectively. A 45 × 0.2 cm i.d. column packed with 5% OV-101 on 120–140-mesh Chromosorb WHP was used. Temperatures were column 175 °C and inlet 200 °C. The nitrogen carrier gas flow was 30 mL/min.

A ^{63}Ni electron capture detector and a 240 × 0.3 cm column packed with 10% OV-1 on 100–120-mesh Chromosorb WHP were used for the detection of 3,5,6-trichloro-2-pyridinol. The column, inlet, and detector temperatures were 175, 200, and 260 °C, respectively. The nitrogen carrier gas flow was 30 mL/min, and the makeup gas flow to the detector was 8 mL/min.

Quantitation was carried out by peak height comparison of at least three closely matched pairs of sample and standard injections for both chlorpyrifos and 3,5,6-trichloro-2-pyridinol.

Procedure. *Chlorpyrifos in Hay.* Hay samples were chopped in a mechanical food chopper and mixed thor-

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oughly before subsampling. A 25-g subsample was transferred to a 1-qt canning jar equipped with an adapter to fit an Omnimixer. Samples for recovery studies were fortified in these quart jars. The samples were blended with 200 mL of 4:1 hexane–2-propanol at high speed for 3 min. The macerate was vacuum filtered through Whatman No. 1 filter paper. A small amount of additional solvent was used to rinse the mixer spindle and the extraction jar. After filtration the filter cake was soaked with an additional 100 mL of extraction solvent for several minutes before refiltering the sample.

Half of the extract was measured by using a graduate cylinder into a separatory funnel and extracted with two 100-mL portions of 0.5% sodium carbonate to separate the 3,5,6-trichloro-2-pyridinol. Ten milliliters of 20% sodium chloride and 10 mL of 2-propanol were added to effect separation. The aqueous phase was backwashed with two 25-mL portions of chloroform, and the backwashings were added to the organic phase. The combined organic extracts were evaporated to near dryness on a steam bath under an air jet, 15 mL of trimethylpentane was added, and the evaporations were repeated until all traces of 2-propanol were removed. The residue was redissolved in 10 mL of hexane for chromatography on silica gel. A glass column, 1.9 × 20 cm, was first plugged with glass wool and then 1.5 cm of anhydrous sodium sulfate and 25 g of deactivated silica gel (Davidson 950; 60–200 mesh; 10% water) were added. The residue was transferred to the column with several small hexane rinses followed by elution with 100 mL of hexane and then with 100 mL of 10% benzene in hexane which were discarded. The chlorpyrifos was collected by eluting the column with 200 mL of 3% water-saturated diethyl ether in hexane. The column eluate was concentrated to 1.0 mL for samples where the chlorpyrifos concentration was near the sensitivity limit of the method (i.e., 0.05 ppm). Samples with higher residue levels were diluted accordingly.

3,5,6-Trichloro-2-pyridinol in Hay. The sodium carbonate extract obtained earlier was acidified with 6 mL of concentrated hydrochloric acid and extracted with two 25-mL portions of chloroform. The chloroform extract was concentrated to a small volume on a steam bath under an air jet, 10 mL of benzene added, and the concentration step repeated until all of the chloroform was removed. The benzene solution was chromatographed on an acidic alumina column (Woelm; activity grade I; stored at 130 °C). Eleven cubic centimeters of the adsorbent was added to the column (2.2 × 33 cm with a coarse porosity fritted disk and a 200-mL reservoir) and wetted with 10 mL of benzene. Ten cubic centimeters of glass beads was added to the top of the adsorbent bed. The sample residue, dissolved in benzene, was added to the column, and after passage of the solvent, the column was washed with 10 mL of methanol. Both of these washes were discarded. The column was then eluted with 80 mL of 40:60 (v/v) concentrated hydrochloric acid–water. The acid eluate was transferred to a separatory funnel, diluted to 200 mL with water, and extracted with two 25-mL portions of chloroform. The chloroform extract was concentrated to near dryness on a steam bath under an air jet, benzene added, and the concentration step repeated.

The residue was dissolved in 10 mL of benzene for low-level samples, 1.0 mL transferred to a 10-mL volumetric flask, 20 μL of BSA [*N,O*-bis(trimethylsilyl)acetamide] added, and the sample diluted to volume with hexane and analyzed by electron capture gas chromatography. Samples with higher residue levels were dissolved in correspondingly larger quantities of benzene. A known amount of 3,5,6-trichloro-2-pyridinol (usually 0.1 μg) was derivatized at the same time and in the same manner and used to calibrate the gas chromatograph.

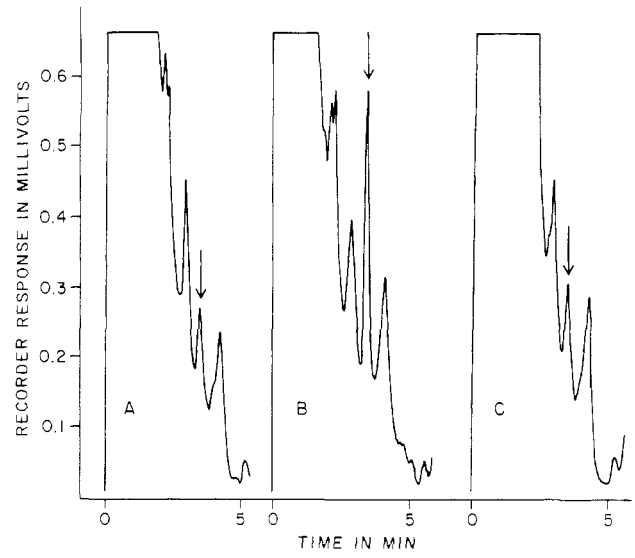

Figure 1. Sample chromatograms of mint oil analyzed for 3,5,6-trichloro-2-pyridinol. (A) Untreated mint oil. (B) Mint oil fortified to 0.5 ppm with 3,5,6-trichloro-2-pyridinol. (C) Mint oil distilled from hay treated at 4.5 kg of AI/ha with chlorpyrifos. Varian 3740 gas chromatograph; electron capture detector; 240 × 0.3 cm column packed with 10% OV-1 on 100–120-mesh Chromosorb WHP at 175 °C; attenuation 16×.

Chlorpyrifos in Mint Oil. One gram of mint oil was transferred to a 25-g deactivated silica gel column, and the column eluted with 120 mL of 3% water-saturated diethyl ether in hexane. The volume of eluate was adjusted for analysis by gas chromatography–flame photometry.

3,5,6-Trichloro-2-pyridinol in Mint Oil. One gram of mint oil was dissolved in 20 mL of benzene and 30 mL of pentane and extracted with two 50-mL portions of 0.5% sodium carbonate. The aqueous phase was backwashed twice with 10 mL of chloroform, and the washes were discarded. The aqueous phase was acidified with 3 mL of concentrated hydrochloric acid, followed by two 25-mL chloroform extractions. The chloroform extract was evaporated to near dryness, and the residue dissolved in benzene, followed by column chromatography, derivatization, and gas chromatography as described for hay.

Method Sensitivity. Untreated mint hay and oil showed no significant response to the analytical method for chlorpyrifos, and there was also no response by untreated hay to the analytical method for 3,5,6-trichloro-2-pyridinol. The sensitivity limits were estimated on the basis of the gas chromatographic response to the analytical standards, base-line noise level, and sample size. The sensitivity of the analytical method was estimated to be 0.02 ppm for hay with a 12.5-g sample and for chlorpyrifos in oil 0.1 ppm with a 1-g sample. However, peaks interfering with the detection of 3,5,6-trichloro-2-pyridinol were observed in samples of untreated mint oil in the range of 0.05–0.2 ppm.

Sample chromatograms of untreated, fortified, and treated mint oils analyzed for 3,5,6-trichloro-2-pyridinol are shown in Figure 1. It was estimated on the basis of the peak heights of the interference that 0.5 ppm could be detected in a 1-g mint oil sample.

RESULTS AND DISCUSSION

This method has been used successfully for three seasons for the determination of residues of chlorpyrifos and its metabolite in mint hay and oil. Typical residue data are

Table I. Residues of Chlorpyrifos and Its Metabolite in Oregon Mint Hay and Oil after Foliar Application[a]

area	days after application	residues, ppm, from application of chlorpyrifos: 2.2 kg of AI/ha chlorpyrifos	2.2 kg of AI/ha metabolite[b]	4.5 kg of AI/ha chlorpyrifos	4.5 kg of AI/ha metabolite[b]
Junction City[c]	0	258	0.74	313	1.13
	7	11.3	0.69	22.4	0.81
	14	8.6	0.70	12.0	0.87
	28	4.7	0.71	5.6	0.88
	90	1.4	0.29	3.6	0.59
	180	0.18	0.07	0.73	0.20
	270	<0.02	<0.02	<0.02	<0.02
	345[e]	<0.02	<0.02	<0.02	<0.02
	345[f]	<0.02	<0.02	<0.02	<0.02
	345[g]	<0.1	<0.5	<0.1	<0.5
Madras[d]	0	125	0.36	331	0.88
	7	8.0	0.73	54	1.52
	14	5.9	0.94	16.1	1.68
	28	0.31	0.08	0.62	0.15
	89[e]	<0.02	<0.02	<0.02	<0.02
	89[f]	<0.02	<0.02	<0.02	<0.02
	89[g]	<0.1	<0.5	0.12	<0.5

[a] One application of Lorsban 4E, 0.48 kg/L, by ground sprayer. [b] 3,5,6-Trichloro-2-pyridinol. [c] Applied Sept 16, 1977; harvested Aug 27, 1978. [d] Applied May 13, 1977; harvested Aug 10, 1977. [e] Mint hay at harvest. [f] Mint hay after distillation. [g] Mint oil distilled from hay.

presented in Table I. The fall application for the control of mint root borer larvae is made after harvest, before much of the regrowth has started, and it is for this reason that high initial residues were found in hay. Chlorpyrifos residues persisted under Oregon winter conditions of cool temperatures and high rainfall for more than 6 months in the mint plants; however, no detectable residues were found in hay or oil at harvest, 11 months after application. When chlorpyrifos was applied in early spring for the control of red-backed cutworm, high initial residues dissipated rapidly. Only ~0.2% of the initial residue was present in mint hay 28 days after treatment. Residues of the metabolite 3,5,6-trichloro-2-pyridinol never reached high levels and were at maximum levels 14–28 days after treatment. Residues of chlorpyrifos and its metabolite were generally absent in spent hay (hay after distillation) and in mint oil.

The reliability of the analytical method was tested by adding known amounts of chlorpyrifos and its metabolite to fresh and spent mint hay and to mint oil, followed by extraction and analysis. The range of fortifications and recoveries is shown in Table II.

The efficiency of the extraction procedure was tested by returning the filter cake from field treated hay samples to the blender and repeating the extraction step. Analysis of the second extract indicated 84% recovery for chlorpyrifos and 85% recovery for 3,5,6-trichloro-2-pyridinol in the first extraction. These results indicated that the majority of the residue was recovered with one extraction. Special studies were carried out to determine the source of errors in the analytical procedure. Triplicate 25-g random samples taken from a single chopped mint hay sample were analyzed separately and yielded a relative standard deviation of 14% for chlorpyrifos and 11% for 3,5,6-trichloro-2-pyridinol. When three aliquots were taken from an extract of the same sample, the relative standard deviation was 10% for chlorpyrifos and 6.7% for the metabolite. The storage stability of chlorpyrifos was studied by adding known amounts of the material in acetone solution to mint hay, allowing the solvent to evaporate, and then storing the samples at −10 °C in the same freezer with the field samples. The range of fortifications was from 0.12 to 1.15 ppm, and the average recovery after a maximum of 35 months of storage was 81%. Mint oil samples were also fortified with known amounts of insecticide and stored at 4 °C with the field samples. The recovery after 32 months of storage was 82%.

Table II. Recovery of Added Chlorpyrifos and Its Metabolite from Untreated Crops

crop	no. of recoveries	level of fortifications, ppm	range of recoveries, %	av recovery, %
		Chlorpyrifos		
fresh mint hay	51	0.16–50	78–115	90
spent mint hay	10	0.05–1.23	78–90	87
mint oil	20	0.11–10	73–104	86
		3,5,6-Trichloro-2-pyridinol		
fresh mint hay	51	0.1–10	66–115	88
spent mint hay	10	0.5–1.23	78–107	92
mint oil	14	0.5–1.0	70–101	85

The sensitivity of the analytical method for 3,5,6-trichloro-2-pyridinol in mint oil was limited because of the interfering peaks on the gas chromatograms which showed apparent residues from 0.05 to 0.2 ppm. A number of schemes were tried in an effort to reduce this interference, including adsorption chromatography, ion-exchange chromatography, high-pressure liquid chromatographic fractionation, and the formation of various derivatives; however, none produced significantly improved chromatograms.

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