Residues of the Nematicide Ethoprop in Mint Hay and Oil

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An analytical method developed for the determination of ethoprop in fresh and spent mint hay included initial extraction with hexane in the presence of anhydrous sodium sulfate, cleanup using charcoal and deactivated Florisil, and quantitation by phosphorus-specific flame photometric gas chromatography. Mint oil samples were introduced directly onto the deactivated Florisil columns, and the ethoprop concentration in the eluate was again determined by gas chromatography. The method is sensitive to 0.002 ppm in mint hay and to 0.05 ppm in mint oil. Recoveries averaged 90% for samples fortified immediately before analysis and 89% for samples stored 4–58 weeks. Residue data from nine locations collected over three seasons showed traces of ethoprop in almost all fresh and spent hay samples ranging from <0.002 to 3.36 ppm. Ethoprop residues in mint oil ranged from <0.05 to 114 ppm, the highest residue being in spearmint oil treated at 13.2 kg of AI/ha 90 days before harvest.

The pesticide ethoprop, *O*-ethyl *S*,*S*-di-*n*-propyl phosphorodithioate, has been shown to be effective for the control of lesion nematodes, *Pratylenchus spp.*, in various crops (Overman, 1976; Johnson et al., 1978). Since these nematodes are also serious pests of mint crops, registration of ethoprop for use on mint would be desirable. However, this depends in part on the availability of a sensitive and specific method for residue determinations and also on the examination of the residual behavior of ethoprop in mint crops.

Analytical methods described for residue analysis of ethoprop in other crops employ hexane or dichloromethane extraction, silicic acid or deactivated Florisil cleanup, and phosphorus-specific flame photometric gas chromatographic quantitation (Argauer and Feldmesser, 1978; Leidy and Sheets, 1980; Hunt et al., 1981).

This paper describes modification of these methods for mint hay and oil analysis involving hexane extraction, charcoal and Florisil cleanups for mint hay, and the use of a deactivated Florisil column for the cleanup of mint oil. Residue data from nine locations in the United States covering 3 years of work are also presented. The analytical method and the residue data presented in this paper should be helpful for obtaining a minor crop registration for ethoprop from the Environmental Protection Agency.

EXPERIMENTAL SECTION

Apparatus and Reagents. A Varian 3700 gas chromatograph equipped with a dual flame photometric detector was used. The identity of residues was confirmed with a Finnigan Model 4023 quadrupole mass spectrometer. Standard laboratory glassware was used throughout the procedure. The analytical grade standard of ethoprop was obtained from the Agrochemicals Division of Rhône-Poulenc, Inc., Monmouth Junction, NJ. All solvents were distilled in glass.

Gas Chromatography. A Varian Aerograph 3700 gas chromatograph equipped with a dual flame photometric detector operated with a 530-nm interference filter for phosphorus analysis was used. The detector temperature was 250 °C with gas flows to the detector of 170, 140, and 80 mL/min for hydrogen, air 1, and air 2, respectively. A 183 × 0.2 cm (i.d.) column packed with 7% OV-17 on 100/120-mesh Chromosorb WHP was used. The column temperature was 220 °C and the injector temperature also 220 °C. The helium carrier gas flow was 23 mL/min. Quantitation was carried out by peak height comparison of at least three closely matched pairs of sample and standard injections. The retention time of ethoprop was about 2.3 min under these conditions.

Gas Chromatography–Mass Spectrometry. A Finnigan Model 4023 quadrupole mass spectrometer with a 4500 source upgrade and data system was used. The source temperature was 140 °C, electron energy was 70 eV, and transfer line and separator oven were at 275 °C. The gas chromatography column employed was a 30 m × 0.25 mm DB-5 capillary column used with a J&W Model I on-column injector. The injector was air-cooled during the injection, and the column was held at 65 °C for 1 min after injection. The column was then programmed to 300 °C at 8 °C/min and held at 300 °C.

Procedure. *Ethoprop in Mint Hay.* Hay samples were chopped in a mechanical food chopper and mixed thoroughly before subsampling. A 100-g subsample was transferred to a 1-qt canning jar equipped with an adaptor to fit an Omnimixer. Recovery samples were fortified with known amounts of ethoprop in these quart jars. Portions of 40 g of anhydrous sodium sulfate and 500 mL of distilled hexane were added, and the sample was macerated at high speed for 3 min. All sample preparation procedures involving hexane must be performed in a hood with adequate ventilation. The macerate was vacuum filtered through Whatman No. 42 filter paper, and 200 mL of additional hexane was used to rinse the mixer spindle, the extraction jar, and the filter cake. The filter cake was then returned to the extraction jar and macerated again with 400 mL of hexane for 2 min. The extract was recovered by filtering with vacuum and the rinsing procedure repeated. All extracts and rinsings were combined and transferred to a round-bottom flask, and the volume was reduced to about 10 mL on a rotary evaporator at 40 °C. This extract was then passed through a 5% water deactivated Florisil column to remove the majority of plant pigments. The deactivated Florisil was prepared as follows: 100–200-mesh Florisil (Floridin Co., Tallahassee, FL) was baked in a muffle furnace for 4 h at 450 °C, stored in an oven at 130 °C until use, and deactivated before use by adding 5% of water by weight and shaking until a homogeneous mixture was obtained no less than 24 h before use. A 1.9 × 50 cm glass chromatography column equipped with a Teflon stopcock was plugged with glass wool and hexane added, followed by 1 cm of anhydrous sodium sulfate, 15.5 g of deactivated Florisil, and 1 cm of anhydrous sodium sulfate. The hexane extract was transferred quantitatively to the column with several small rinses of hexane and the column washed with 50 mL of hexane, discarding the washings. The column was eluted with 140 mL of 20% ethyl acetate in hexane and the eluate concentrated to 2–3 mL on a rotary evaporator at 40 °C. The eluate was then trans-

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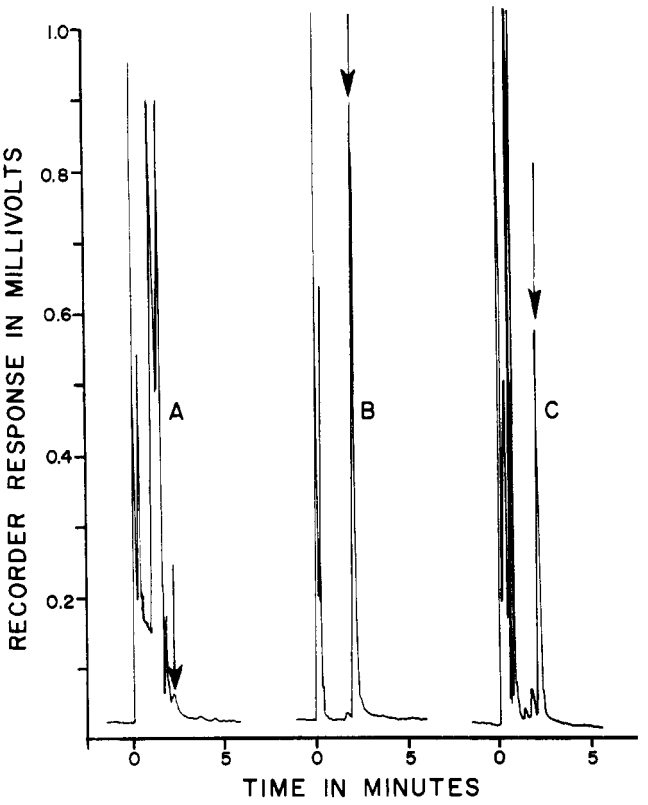

Figure 1. Sample chromatograms of mint oil analyzed for ethoprop: (A) untreated mint oil; (B) mint oil fortified to 5.0 ppm with ethoprop; (C) mint oil distilled from hay treated with 6.6 kg of AI/ha ethoprop. Conditions: Varian 3700 gas chromatograph; phosphorus-specific flame photometric detector; 183 × 0.2 cm (i.d.) column packed with 7% OV-17 on 100/120-mesh Chromosorb WHP at 220 °C.

ferred quantitatively to a calibrated test tube and the volume adjusted for gas chromatography.

To obtain better cleanup, the 1986 samples were treated with charcoal before passage through the Florisil column. Portions of 4 g of charcoal (Darco G-60, 20–40 mesh; Matheson, Coleman and Bell, Norwood, OH) were added to the hexane extract before concentration, the solution was mixed with a magnetic stir bar, and the sample was filtered through Whatman No. 1 filter paper into a 200-mL round-bottom flask. The residual charcoal was rinsed with hexane and then with 50 mL of chloroform. The filtrate and the rinses were concentrated to about 10 mL on a rotary evaporator at 40 °C for Florisil column chromatography.

Ethoprop in Mint Oil. A 2-g portion of mint oil was weighed into a 10-mL flask and transferred to a Florisil column with a disposable pipet followed by five hexane rinses of the flask that were also transferred to the column. The procedure for the hay was then followed.

Limit of Detection. Traces of ethoprop were found in untreated samples of mint hay and oil from several locations whereas untreated samples from other locations were completely free of residues. The improved cleanup developed for the 1986 samples did not reduce the small residues of ethoprop found in some untreated samples. It was assumed for these reasons that the residues found were actual residues of ethoprop caused by drift and not due to interfering materials in the crop. Drift is always a hazard with small experimental plots located closely together. The sensitivity limits of the analytical methods were therefore estimated on the basis of the gas chromatographic response to the analytical standards, base-line noise level, and sample size. The sensitivity limit of the analytical method was estimated to be 0.002 ppm for mint hay with a 100-g sample and 0.05 ppm for mint oil with a 2-g sample.

Sample chromatograms of untreated, fortified, and treated mint oils were shown in Figure 1.

Field Plots. Experimental plots were established in all major mint growing areas in the United States: Oregon, Washington, Indiana, Michigan, and Wisconsin. Plot sizes ranged from 9 to 1115 m^2, and the plots were replicated two to eight times. The 6EC (0.72 kg of AI/L) emulsifiable concentrate formulation was applied by small CO_2-operated backpack sprayers or by tractor-mounted boom in 31–58 L of water/ha, and the 15G (0.15 kg of AI/kg) granular formulation was applied by granular applicator or by Gandy spreader. The pesticide was incorporated into the soil by raking, plowing, disking, harrowing, or irrigation. Most applications were made in the spring, about 3 months before harvest; the only exceptions were the Redmond, OR, 1984 plots where the treatment to harvest interval was about 4.5 months and the Iola, WI, 1985 plots which were treated in the fall, 11 months before harvest. All plots were harvested at the time of commercial harvest in the area. The random samples were frozen immediately and shipped frozen to the laboratory where they were stored at −21 °C until analysis.

Distillation of Mint Oil. Because of the use of small experimental plots, insufficient amounts of hay were available for the use of field stills and the oil had to be recovered by a laboratory still. The hay was received from the field plots frozen and chopped with a food chopper in the laboratory, and 3–4 kg of hay was packed into a 12-L glass distillation flask. Water was added to the flask at the rate of 1.6 mL/g of hay and the flask connected to a water-cooled condenser. The flask was heated with a heating mantle and the distillate collected at the rate of 0.25 mL of distillate/g of hay. The oil was separated from the aqueous distillate in a separatory funnel and stored at 4 °C until analysis. The hay after distillation (spent hay) was stored frozen until analysis.

RESULTS AND DISCUSSION

The method described has been successfully used for three seasons for the determination of residues of ethoprop in mint hay and oil. Typical residue data from nine locations are presented in Table I. These data show that traces of ethoprop were present in all fresh and spent hay samples with the exception of the 1984 Redmond, OR samples. The stability of ethoprop in mint hay is illustrated by the 1985 Iola, WI, samples where traces of residue were found in the hay harvested more than 11 months after treatment. The residues resulting from the application of emulsifiable concentrate or granular formulations were similar, and residues found in spent hay were comparable in magnitude to the residues in fresh hay. The highest hay residues were found in spearmint samples from the state of Washington.

All oil samples distilled from the hay also contained detectable residues except the 1984 Redmond, OR, oil treated at the lower applications rate. It is noteworthy that the oils from the 1985 Iola, WI, plots contained residues comparable in magnitude to residues in oils distilled from hay treated only 3 months before harvest. As with the hay, the highest residues were found in spearmint oils from the state of Washington.

The reliability of the analytical method was tested by adding known amounts of ethoprop to fresh and spent hay and to mint oil, followed by extraction and analysis. The ranges of fortifications and recoveries are shown in Table II, indicating an overall average recovery of 90%.

The storage stability of ethoprop was studied by adding known amounts of the material in acetone solution to mint hay, allowing the solvent to evaporate, and storing the samples at −21 °C in the same freezer with field samples. Mint oils were also fortified with an acetone solution of ethoprop and then stored at 4 °C. The results of the storage studies given in Table III show an overall average recovery of 89%; however, the range of individual recoveries varied considerably, which may be due to inadequate mixing of the samples before storage or to poor subsampling.

The identity of ethoprop residues in some samples was confirmed by gas chromatography–mass spectrometry. The

Table I. Residues of Ethoprop in Mint Hay and Oil from Nine Locations[a]

location	year	crop	treatment [no. of applicn, kg AI/ha, applicn form]	days after last applicn	residue, ppm		
					fresh hay[b]	spent hay[c]	oil[d]
Redmond, OR[e]	1984	peppermint	1, 6.6, EC	135	<0.002	<0.002	<0.05
Redmond, OR[e]	1984	peppermint	1, 13.2, EC	135	<0.002	<0.002	0.05
Madras, OR[f]	1984	peppermint	1, 6.6, gran	83	0.003	0.002	0.38
Madras, OR[f]	1984	peppermint	1, 13.2, gran	83	0.011	0.003	0.66
Madras, OR[g]	1984	peppermint	2, 6.6, EC	83	0.009	0.005	1.02
Madras, OR[g]	1984	peppermint	2, 13.2, EC	83	0.012	0.024	2.19
Sunnyside, WA[h]	1984	spearmint	2, 6.6, EC, gran	54	0.52	0.28	51
Sunnyside, WA[h]	1984	spearmint	2, 13.2, EC, gran	54	0.97	0.38	66
Iola, WI[i]	1985	peppermint	1, 6.6, gran	324	0.003	0.003	0.19
Iola, WI[i]	1985	peppermint	1, 13.2, gran	324	0.004	0.004	0.57
Albany, OR[j]	1986	peppermint	1, 6.6, EC	89	0.009	0.004	0.48
Albany, OR[j]	1986	peppermint	1, 13.2, EC	89	0.005	0.006	0.49
Rensselaer, IN[k]	1986	peppermint	1, 6.6, EC	91	0.004	0.005	0.46
Rensselaer, IN[k]	1986	peppermint	1, 13.2, EC	91	0.006	0.004	0.51
Madison, WI[l]	1986	peppermint	1, 6.6, gran	90	0.051	0.030	5.17
Madison, WI[l]	1986	peppermint	1, 13.2, gran	90	0.042	0.031	5.51
Owasso, MI[m]	1986	spearmint	1, 6.6, gran	94	0.007	0.093	1.31
Owasso, MI[m]	1986	spearmint	1, 13.2, gran	94	0.015	0.014	1.82
Prosser, WA[n]	1986	spearmint	1, 6.6, EC	90	0.23	0.11	7.46
Prosser, WA[n]	1986	spearmint	1, 13.2, EC	90	3.36	1.93	114

[a] Residues not corrected for recovery. [b] Mint hay at harvest. [c] Mint hay after distillation. [d] Mint oil distilled from hay. [e] Emulsifiable concentrate formulation applied April 20, 1984, by backpack sprayer. Harvested Sept 2, 1984. [f] Granular formulation applied June 7, 1984, by granular applicator. Harvested Aug 29, 1984. [g] Emulsifiable concentrate formulation applied Oct 28, 1983, and June 7, 1984, by field sprayer. Harvested Aug 29, 1984. [h] Emulsifiable concentrate formulation applied May 5, 1984, by backpack sprayer and granular formulation applied Aug 16, 1984, by granular applicator. Harvested Oct 9, 1984. [i] Granular formulation applied Sept 15, 1984, by granular applicator. Harvested Aug 22, 1985. [j] Emulsifiable concentrate formulation applied May 4, 1986, by plot sprayer. Harvested Aug 1, 1986. [k] Emulsifiable concentrate formulation applied April 8, 1986, by tractor-mounted boom. Harvested July 8, 1986. [l] Granular formulation applied April 22, 1986, by Gandy spreader. Harvested July 23, 1986. [m] Granular formulation applied May 9, 1986, by Gandy spreader. Harvested Aug 11, 1986. [n] Emulsifiable concentrate formulation applied May 30, 1986, by plot sprayer. Harvested Aug 28, 1986.

Table II. Recovery of Added Ethoprop from Untreated Crops

crop	no. rec	level of fortifcn, ppm	av rec, %	rec range, %	SD, %
fresh hay	26	0.02–1.06	88	51–113	13
spent hay	22	0.02–1.06	83	49–101	18
oil	36	0.04–53	101	60–126	13

Table III. Storage Stability of Ethoprop in Mint Hay and Oil

crop	storage,[a] days	no. samples	level of fortifcn, ppm	av rec, %	rec range, %	SD, %
fresh hay	57–331	18	0.05–10	91	68–140	16
spent hay	46–193	18	0.05–10	82	71–91	7
oil	29–406	32	0.05–1.13	91	54–114	12

[a] Hay samples stored at −21 °C, oil samples at 4 °C.

mass spectra of an ethoprop standard and those of three field samples are shown in Figure 2. The mass number data provided by the GC–MS system for the treated samples corresponded to the mass spectrum of the ethoprop standard that showed prominent peaks at masses 65, 74, 93, 126, 139, 168, 200, and 242.

It was observed that when peak heights obtained with standard solutions prepared in hexane or toluene were used to calculate recoveries, the resulting recoveries were frequently high (~120%). More realistic recoveries were obtained when the standard solutions were prepared in extracts of untreated crops, and therefore the results reported in this study are based on standards so prepared. It is speculated that some breakdown of the nematicide prepared in a solvent solution may occur during gas chromatography and that the plant extractives offer some protection by coating the more active sites on the gas chromatographic column.

The mint plants contain on the average approximately 0.5% of oil, so that a 200-fold concentration of pesticide residue is possible if all the pesticide distills with the oil. Our distillation data showed that about 0.3% of the weight of the hay was recovered as oil, indicating a possible 300-fold concentration of residue in oil. A comparison of the ethoprop residues in fresh hay and oil shows an average concentration factor of about 100-fold (range 32–187-fold), indicating that ethoprop was quite stable under our distillation conditions and that it was concentrated in the oil. Previous results obtained in this laboratory show that the organophosphorus insecticides naled (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate) and phorate (*O,O*-diethyl *S*-[(ethylthio)methyl] phosphorodithioate) were also concentrated into the oil to some extent (Starr et al., 1963; Kiigemagi et al., 1973), as were the chlorinated hydrocarbon insecticides (Starr et al., 1963). This is in contrast to the carbamate insecticides where oils distilled from treated hays were free of residues (Kiigemagi et al., 1973, 1982, 1984).

All the oils analyzed in this study were recovered from the hay in a laboratory still, and our past experiences have shown that pesticide residues in mint oils recovered with laboratory or pilot stills were consistently higher than those found in oils obtained with commercial stills (Starr et al., 1963). This is because we have found it difficult to duplicate field conditions in the laboratory. The laboratory still was an all-glass apparatus while the commercial stills are made of steel; also, superheated steam is used in the field whereas the steam generated in the laboratory still was at 100 °C. The oil is separated from the aqueous distillate in a steel apparatus in a commercial operation as compared again to a glass separatory funnel in the laboratory. The milder conditions used in a laboratory operation make it possible for a higher proportion of the residue present to survive the distillation.

It should be kept in mind when pesticide residue data are evaluated in mint oil that it is customary in the essential oil industry to refine raw oils and to blend oils from

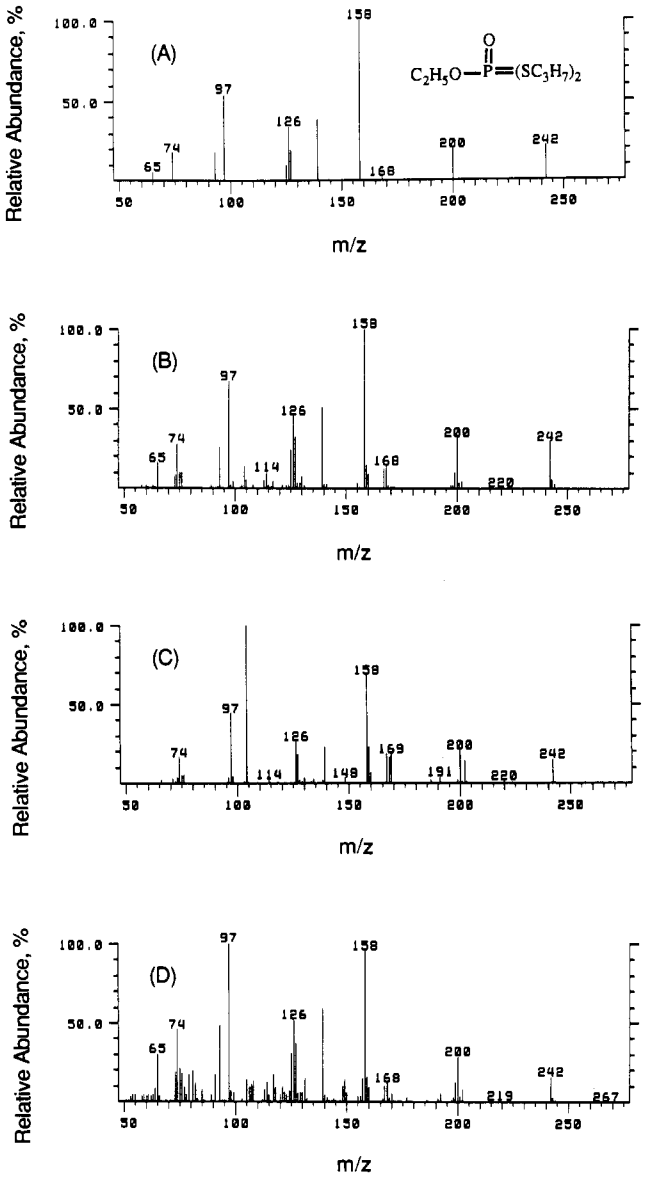

Figure 2. Electron impact spectra of ethoprop: (A) standard, 0.4 μg of ethoprop; (B) isolated from fresh hay sample 821, 3.36 ppm ethoprop; (C) isolated from spent hay sample 821, 1.83 ppm ethoprop; (D) isolated from mint oil sample 42, 66 ppm ethoprop. Conditions: Finnigan 4023 instrument; ionization voltage, 70 eV.

different regions to obtain uniform and desirable flavor characteristics of the oils used as flavoring agents. These practices tend to reduce the pesticide content of the oil, and since the analyses reported here were performed on raw oils obtained with a laboratory still, it would be expected that the residue in commercial oils would be considerably less. In its commercial use, mint oil undergoes a 100–1000-fold dilution, which would further reduce ethoprop residues in the finished product.

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