FDA, Detroit, MI, provided us with additional standard agricultural chemicals, as well as a preprint of his related work. Carl Selavka at Northeastern University provided certain technical assistance and encouragement during these studies. X.-D. Ding is a Visiting Chinese Scholar from the Chinese Academy of Sciences, Beijing, China. We are grateful to the People's Republic of China and the Chinese Academy of Sciences for providing X.-D. Ding with the opportunity to collaborate with his colleagues at Northeastern University.

Registry No. Guthion, 86-50-0; *o,p'*-kelthane, 10606-46-9; malathion, 121-75-5; lorox, 330-55-2; famphur, 52-85-7; EPN, 2104-64-5; imidan, 732-11-6; ethyl guthion, 2642-71-9; coumaphos, 56-72-4; leptophos, 21609-90-5; supracide, 950-37-8; pirimiphos ethyl, 23505-41-1; parathion, 56-38-2; ethion, 563-12-2; abate, 3383-96-8; phosalone, 2310-17-0; thimet, 298-02-2; dioxathion, 78-34-2; dasanit, 115-90-2; mocap, 13194-48-4.

LITERATURE CITED

Das, K. G., Ed. "Pesticide Analysis"; Marcel Dekker: New York, 1981.
Frei, R. W.; Lawrence, J. F., Eds. "Chemical Derivatization in Analytical Chemistry. Volume 1: Chromatography"; Plenum Press: New York, 1981; Chapter 1.
Green, C.; Hansen, T. J.; Iwaoka, W. T.; Tannenbaum, S. R. "Proceedings of the Second International Symposium on Nitrite in Meat Products"; PUDOC Publishers, Wageningen, Holland, 1977; p 145.
Harvey, J., Jr.; Zweig, G., Eds. "Pesticide Analytical Methodology"; American Chemical Society: Washington, DC, 1980; ACS Symp. Ser. No. 136.
Krull, I. S.; Bratin, K.; Shoup, R. E.; Kissinger, P. T.; Blank, C. L. *Am. Lab. (Fairfield, Conn.)* **1983**, *15* (2), 57.
Krull, I. S.; Ding, X.-D.; Selavka, C.; Bratin, K.; Forcier, G. *J. Forensic Sci.* **1984**, in press.
Krull, I. S.; Lankmayr, E. P. *Am. Lab. (Fairfield, Conn.)* **1982**, *14* (5), 18.
Lawrence, J. F., Ed. "Liquid Chromatography in Environmental Analysis"; The Humana Press: Clifton, NJ, 1984; Chapter 3.
Lawrence, J. F.; van Buuren, C.; Brinkman, U. A. Th.; Frei, R. W. *J. Agric. Food Chem.* **1980**, *28*, 630.
Lefevre, M. F.; Frei, R. W.; Scholten, A. H. M. T.; Brinkman, U. A. Th. *Chromatograpia* **1982**, *15*, 459.
Moye, H. A. *J. Chromatogr. Sci.* **1975**, *13*, 268.
Nondek, K.; Brinkman, U. A. Th.; Frei, R. W. *Anal. Chem.* **1983**, *55*, 1466.
Papadopoulou-Mourkidou, E.; Iwata, Y.; Gunther, F. A. *J. Liq. Chromatogr.* **1981**, *4* (9), 1663.
"Pesticide Analytical Manual"; U.S. Food and Drug Administration: Washington, DC, 1977; Vol. I, Section 212.101.
"Pesticide Analytical Manual"; U.S. Food and Drug Administration: Washington, DC, 1982; Vol. I, Section 211.13f.
Scholten, A. H. M. T.; Brinkman, U. A. Th.; Frei, R. W. *Anal. Chim. Acta* **1980**, *114*, 137.
Scholten, A. H. M. T.; Brinkman, U. A. Th.; Frei, R. W. *Anal. Chem.* **1982**, *54*, 1932.
Sherwood, G. A.; Johnson, D. C. *Anal. Chim. Acta* **1981**, *129*, 101.
Shoup, R. W., Ed. "Recent Reports on Liquid Chromatography/Electrochemistry"; Bioanalytical Systems, Inc.: West Lafayette, IN, 1982.
Shuker, D. E. G.; Tannenbaum, S. R. *Anal. Chem.* **1983**, *55*, 2152.
Snider, B. G.; Johnson, D. C. *Anal. Chim. Acta* **1979**, *106*, 1.
Walters, S. M. *J. Chromatogr.* **1983**, *259*, 227.
Xie, K.-H.; Colgan, S.; Krull, I. S. *J. Liq. Chromatogr.* **1983**, *6* (S-2), 125.

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Determination of Oxamyl Residues in Peppermint Hay and Oil Using a Radioisotope Dilution Technique

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Procedures are described for the determination of the nematocide oxamyl [methyl *N',N'*-dimethyl-*N*-[(methylcarbamoyl)oxy]-1-thiooxamimidate] in peppermint hay and oil. After extraction of the hay with ethyl acetate, oxamyl residues are cleaned up on an alumina column and hydrolyzed with alkali to the oxime (methyl *N',N'*-dimethyl-*N*-hydroxy-1-thiooxamimidate), followed by additional cleanup on silica gel, formation of a trimethylsilyl ether derivative, and quantitation by sulfur specific flame photometric gas chromatography. Peppermint oil is diluted with toluene and extracted with water, followed by hydrolysis, silica gel cleanup, derivative formation, and gas chromatography as described for hay. A radioisotope dilution method was used to compensate for low recoveries. The method is sensitive to 0.05 ppm in peppermint hay and to 0.1 ppm in peppermint oil. Although low residues of oxamyl were found in fresh peppermint hay at harvest, no residues were detected in peppermint oil.

The nematocide oxamyl [methyl *N',N'*-dimethyl-*N*-[(methylcarbamoyl)oxy]-1-thiooxamimidate] has shown considerable promise for the control of the nematode *Longidorus elongatus* in mint fields of Oregon. Registration of oxamyl for the control of this pest depends in part on the availability of a sensitive and specific method for residue determinations in this crop.

The first analytical method for oxamyl was described by Holt and Pease (1976), who hydrolyzed oxamyl to its oxime and determined this more stable and volatile derivative by sulfur-specific flame photometric gas chromatography. A spectrophotometric method was presented by Singhal et al. (1977) and HPLC was employed by Thean et al. (1978), Davis et al. (1978), and Chiba et al. (1983).

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Chapman and Harris (1979) derivatized the hydrolysis product of oxamyl with BSA followed by sulfur-selective gas chromatography. Greenberg (1981) used an alumina column for cleanup of vegetable extracts and quantitated the residues by direct gas chromatography of oxamyl on a Carbowax 20M column.

A method for oxamyl in peppermint hay and oil using sulfur-specific gas chromatography and radioisotope dilution is presented in this report. The use of the radioisotope dilution technique in pesticide residue analysis was first described by Redeman and Meikle (1958) for the analysis of dalapon in various crops. These authors believed that this technique could be used to solve many problems in pesticide residue analysis and recommended that other workers use radioisotope dilution to solve their problems. Despite this recommendation, the radioisotope dilution technique has found limited use in pesticide residue analysis. Angoso et al. (1973) employed this technique for the analysis of oranges for fenthion, Åkerblom (1974) for the analysis of diquat in rape seed, and Kohli et al. (1979) for the analysis of polychlorinated biphenyls.

EXPERIMENTAL SECTION

Apparatus and Reagents. A Varian 204 gas chromatograph equipped with a flame photometric detector was used. A Packard Model 3330 Tri-Carb liquid scintillation spectrometer was used for radioactivity measurements. Standard laboratory glassware was used throughout the procedure. The analytical-grade standards of oxamyl and oxime were obtained from the Biochemicals Department, E. I. du Pont de Nemours and Co., Inc., Wilmington, DE. The [1-^{14}C]oxamyl with a specific activity of 5.6 μCi/mg and a radiochemical purity of >99%, as determined by the donor, was also supplied by Du Pont. [^{14}C]Toluene with an activity of 8.95×10^5 dpm/g was obtained from Amersham Corp., Arlington Heights, IL, and was used as an internal standard to determine the quenching of each sample. BSA [*N*,*O*-bis(trimethylsilyl)acetamide] was supplied by Pierce Chemical Co., Rockford, IL. All solvents were distilled in glass.

Gas Chromatography. A Melpar Model FPD 100 flame photometric detector operated with the 394-nm interference filter for sulfur was used. The detector temperature was 175 °C with gas flows to the detector of 100, 45, and 25 mL/min for hydrogen, air, and oxygen, respectively. A 60 × 0.2 cm i.d. glass column packed with 20% OV-101 on 100–120-mesh Chromosorb WHP was used. The column temperature was 150 °C, injector temperature 180 °C, and nitrogen carrier gas flow 30 mL/min. The instrument was vented for 1.5 min after each injection. The effluent from the gas chromatograph was passed through a venting system to the roof of the building and then to the outside atmosphere.

Quantitation was carried out by using the power equation

$$c = ah^n \tag{1}$$

where c is the analyte concentration, h is the peak height, a is a constant, and n is the power of the response. n varied from 0.6 to 0.8 during the course of this work. The amount of the oxime present in the unknowns was calculated from each day's data with a Hewlett-Packard 41C programmed calculator. The final concentration of oxamyl was corrected for the molecular weight difference between oxamyl and oxime (1.35).

Gas Chromatography–Mass Spectrometry. A Finnigan Model 4023 quadrupole mass spectrometer with 4500 source upgrade and data system was used. The gas chromatographic column was 183 × 0.2 cm i.d. glass packed with 7% OV-101 on 100–120-mesh Chromosorb WHP. The helium carrier gas was diverted from the mass spectrometer for 30 s after each injection. Injection port and column temperatures were 180 and 170 °C, respectively, and the transfer and separator oven was at 200 °C. The ion source was operated at 70 eV and 190 °C.

Radioactivity Measurements. Aliquots of the extracts to be measured (usually 0.1 mL) were transferred to scintillation vials, and 10 mL of scintillation liquid (0.004 g/L PPO and 0.00005 g/L POPOP in toluene) was added. Each sample was prepared in duplicate and was counted for 50 min in a liquid scintillation spectrometer. A known amount of [^{14}C]toluene was then added to each sample, and the samples were recounted to determine the amount of quenching. The results for each sample were corrected for quenching, and the amount of oxamyl in each sample was calculated according to the formula

$$m = m_0\left(\frac{s_0}{s} - 1\right) \tag{2}$$

where s_0 is the specific activity of [^{14}C]oxamyl added, s is the specific activity of the final solution, and m_0 and m are the masses of the labeled and unlabeled oxamyl in the sample, respectively. Specific activity of the final solution was calculated from $s = a/m'$, where a is the activity measured and m' is the total mass of oxamyl measured by gas chromatography.

Procedure. *Oxamyl in Peppermint Hay.* Hay samples were chopped in a mechanical food chopper and mixed thoroughly before subsampling. A 30-g subsample was transferred to a 1-qt canning jar equipped with an adaptor to fit an omnimixer. A known amount of [^{14}C]oxamyl was added to each sample, and the recovery samples were also fortified with unlabeled oxamyl in these quart jars. The samples were blended with 300 mL of ethyl acetate at high speed for 5 min. The macerate was vacuum filtered through Whatman No. 1 filter paper, and 90 mL of additional ethyl acetate was used to rinse the mixer spindle, the extraction jar, and the filter cake.

An aliquot of the extract corresponding to 20 g of hay was transferred into a round-bottom flask and concentrated to about 10 mL on a rotary evaporator at 40 °C. Ten milliliters of toluene was added, and the evaporations and toluene additions were repeated 3 times. This extract was then passed through a deactivated alumina column to remove the majority of plant pigments. A glass chromatography column, 1.9 × 50 cm, was first plugged with glass wool and then 1.5 cm of anydrous sodium sulfate, 10 g of 10% deactivated alumina (Bakers Analyzed Reagent neutral alumina, activity grade I, baked at 450 °C overnight and stored at 130 °C, 10% water deactivated before use), and 1.5 cm of anhydrous sodium sulfate were added. The residue was transferred to the column with several small rinses of 20% acetone in hexane and the column washed with 40 mL of this solvent discarding the washings. The column was eluted with 50 mL of 60% acetone in hexane. The eluate was transferred to a round-bottom flask, 50 mL of water added, and the organic solvent removed on a rotary evaporator at 40 °C. The aqueous extract was transferred to a separatory funnel, acidified with 1 mL of concentrated hydrochloric acid, and washed with two 50-mL portions of hexane, discarding the hexane layers. The solution was then made strongly basic with 15 mL of 1 N sodium hydroxide (pH $\simeq$ 12) and shaken with two 25-mL portions of chloroform, discarding the chloroform layers. The aqueous phase was transferred to a round-bottom flask and refluxed under a water-cooled

condenser for 10 min. The solution was allowed to cool and transferred to a separatory funnel, 1 mL of 1 N sodium hydroxide was added, and the solution was shaken with two 25-mL portions of chloroform, which were discarded. The aqueous solution was made slightly acidic (pH 4–6) with glacial acetic acid, 15 g of sodium chloride was added, and the solution was extracted with four 50-mL portions of 9:1 ethyl acetate–methanol. The organic layer was filtered through anhydrous sodium sulfate into a round-bottom flask and concentrated to about 10 mL on a rotary evaporator at 40 °C. Ten milliliters of toluene was added, and the evaporations and toluene additions were repeated 3 times. The residue was passed through a deactivated silica gel column for additional cleanup. A glass chromatography column, 1.9 × 50 cm, was first plugged with glass wool and then 1.5 cm of anhydrous sodium sulfate, 10 g of 10% deactivated silica gel (Davidson 950, stored at 130 °C, 10% water deactivated before use), and 1.5 cm of anhydrous sodium sulfate were added. The sample was transferred to the column with several small rinses of toluene, and the column was washed with 50 mL of toluene and 100 mL of 40% diethyl ether in hexane, discarding the washings. The column was eluted with 200 mL of 80% diethyl ether in hexane and the eluate concentrated to a small volume on a rotary evaporator at 40 °C. Ten milliliters of toluene was added, and the evaporations and toluene additions were repeated 3 times. The final toluene solution was filtered through anhydrous sodium sulfate into a graduated centrifuge tube and an aliquot taken for radioactivity measurement. The remaining solution was concentrated to about 0.4 mL under an air jet at room temperature, 0.1 mL of BSA added, and the solution allowed to stand overnight at room temperature. Alternatively, the solution was heated in a capped tube for 1 h at 60 °C. Sample volume was then adjusted for gas chromatography.

Oxamyl in Peppermint Oil. Ten-gram samples of oil were dissolved in 100 mL of toluene and a known amount of [^{14}C]oxamyl was added to each sample. The recovery samples were also fortified at this point with unlabeled oxamyl. This solution was extracted with two 50-mL portions of deionized water and the pH of the aqueous phase adjusted to about 12 with 1 N sodium hydroxide. The aqueous phase was shaken with two 25-mL portions of chloroform, which were discarded, and the sample was transferred to a round-bottom flask and refluxed under water-cooled condenser for 10 min. The aqueous extract was cooled, shaken with chloroform, and extracted with 9:1 ethyl acetate–methanol, the organic solvent was concentrated to a small volume on a rotary evaporator, 10 mL of toluene was added, the evaporations and toluene additions were repeated 3 times, the extract was cleaned up on a silica gel column, and the BSA derivative was formed as described for hay. An aliquot of the final solution was also taken for the determination of radioactivity.

Limit of Detection. No apparent oxamyl residues were detected in untreated peppermint hay and oil. [^{14}C]Oxamyl was added to all samples; thus all chromatograms showed a peak corresponding to 0.05–0.1 ppm of oxamyl. The limit of detection was defined as the lowest concentration of oxamyl that gave an increase of this base peak that could be reliably detected. For instance, the average peak height for mint oil samples fortified to 0.1 ppm with labeled oxamyl was 2.2 ± 0.35 cm, whereas the peak heights of samples fortified with both labeled and unlabeled oxamyl at the 0.1-ppm level averaged 4.3 ± 0.76 cm, an almost 2-fold increase in peak height that could be easily detected. Similar results were obtained with hay.

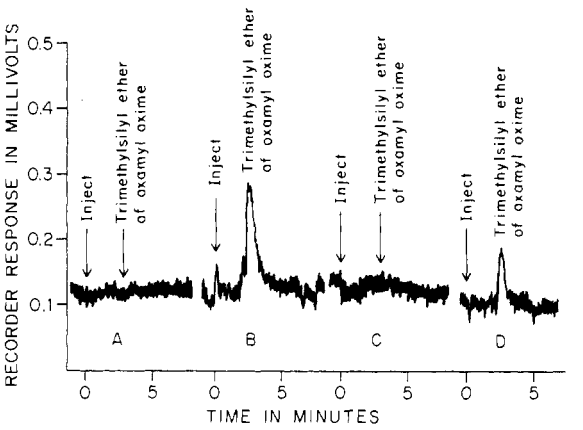


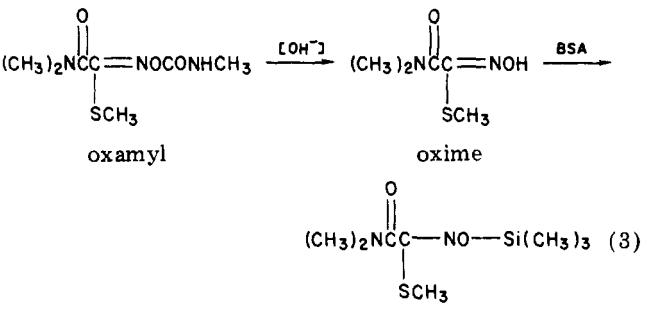
Figure 1. Sample chromatograms of peppermint oil analyzed for oxamyl. (A) Untreated peppermint oil. (B) Peppermint oil fortified to 0.1 ppm with unlabeled oxamyl and to 0.1 ppm with labeled oxamyl. (C) Peppermint oil distilled from hay treated 2 times with 2.2 kg of AI/ha oxamyl. (D) Peppermint oil fortified to 0.1 ppm with labeled oxamyl. Varian 204 gas chromatograph; sulfur-specific flame photometric detector; 60 × 0.2 cm i.d. column packed with 20% OV-101 on 100–120-mesh Chromosorb WHP at 150 °C.

On this basis it was estimated that the limit of detection was 0.05 ppm with a 20-g sample for hay and 0.1 ppm with a 10-g sample for oil.

Sample chromatograms of untreated, fortified, and treated peppermint oil are shown in Figure 1. The retention time for the BSA derivative of oxime was about 2.8 min at 150 °C.

RESULTS AND DISCUSSION

A specific and sensitive method was required for obtaining oxamyl residue data in peppermint hay and oil. The method developed included the hydrolysis of the carbamate (oxamyl) to its oxime (methyl *N'*,*N'*-di-



methyl-*N*-hydroxy-1-thiooxamimidate), which was then derivatized with BSA to form the trimethylsilyl ether to increase the sensitivity of the method. Specificity was obtained by using sulfur-specific flame photometric gas chromatography. Because of low recoveries, a radioisotope dilution technique was used to correct the results.

This method has been used successfully for the determination of residues of oxamyl in field samples received from experimental plots. Typical residue data are presented in Table I. The data show that the oxamyl residues in fresh peppermint hay at harvest were low, even when a total of 4.4 kg of oxamyl was applied per ha and the last application was only 49 days before harvest. Treatments with the granular formulation resulted in slightly higher residues than the treatments with the emulsifiable formulation. Only occasional traces were found in hay after distillation while peppermint oil, the product used for

Table I. Residues of Oxamyl in Oregon Peppermint Hay and Oil

treatment	days after last appl.	residue, ppm fresh hay[e]	spent hay[f]	oil[g]
2 appl, 1.1 kg of AI/ha, gran[a]	49	0.10	<0.05	<0.1
2 appl, 1.1 kg of AI/ha, gran[b]	49	0.22	<0.05	<0.1
2 appl, 1.1 kg of AI/ha, EC[c]	49	<0.05	<0.05	<0.1
2 appl, 1.1 kg of AI/ha, EC[d]	49	0.06	<0.05	<0.1
2 appl, 2.2 kg of AI/ha, gran[a]	49	0.35	<0.05	<0.1
2 appl, 2.2 kg of AI/ha, gran[b]	49	0.44	0.11	<0.1
2 appl, 2.2 kg of AI/ha, EC[c]	49	0.06	0.10	<0.1
2 appl, 2.2 kg of AI/ha, EC[d]	49	0.16	<0.05	<0.1

[a] Granular formulation applied Nov 23, 1977, and June 16, 1978, by broadcasting by hand. Harvested Aug 4, 1978. [b] Granular formulation applied Feb 27 and June 16, 1978, by broadcasting by hand. Harvested Aug 4, 1978. [c] Emulsifiable concentrate formulation applied Nov 23, 1977, and June 16, 1978, by hand-operated sprayer. Harvested Aug 4, 1978. [d] Emulsifiable concentrate formulation applied Feb 27 and June 16, 1978, by hand-operated sprayer. Harvested Aug 4, 1978. [e] Peppermint hay at harvest. [f] Peppermint hay after distillation. [g] Peppermint oil distilled from hay.

Table II. Recovery of Added Oxamyl from Peppermint Hay and Oil

crop	no. of recoveries	level of fort., ppm	average recovery, %	range of recoveries, %	SD, %
Gas Chromatography[a]					
fresh hay	10	0.1	59	51-68	5.7
spent hay	16	0.1	57	48-70	6.9
oil	61	0.1	68	48-94	11.1
Radiometry					
fresh hay	18	0.1	63	55-70	4.4
spent hay	18	0.1	63	50-70	6.4
oil	59	0.1	75	53-115	12.8

[a] Recoveries not corrected by the radioisotope dilution technique.

Table III. Recovery of [1-^{14}C]Oxamyl during Analysis

steps in analysis	recovery, %[a] fresh hay[b]	spent hay[c]	oil[d]
extraction	95 ± 2.8	94 ± 5.6	93 ± 3.8
alumina chromatography	92 ± 5.4	93 ± 4.9	[e]
hydrolysis	93 ± 3.4	93 ± 3.7	92 ± 5.5
ethyl acetate:methanol extraction	78 ± 5.7	81 ± 4.1	77 ± 4.5
silica gel chromatography	71 ± 5.9	70 ± 9.0	71 ± 7.0
overall recovery	64 ± 4.8	63 ± 7.2	66 ± 6.3

[a] Average and standard deviation. [1-^{14}C]Oxamyl added to hay and oil before extraction. Recovery determined after each step by using a liquid scintillation counter. Percent recovery based on initial fortification. [b] Average of 13 analyses. [c] Average of 11 analyses. [d] Average of 10 analyses. [e] Not used.

Table IV. Partitioning of Oxime Fragment of Oxamyl into Ethyl Acetate-Methanol (9:1) from an Aqueous Solution at Various pHs

pH of aqueous phase	recovery in solvent, %[a] 1st extr	2nd extr	3rd extr	4th extr	5th extr	total
1.8	44.4	21.6	7.5	4.1	1.4	79.0
4.0	48.6	21.6	8.7	2.9	0.1	81.9
6.8	44.4	20.6	10.8	3.2	1.9	80.9
7.0	51.6	16.9	9.1	3.0	0.8	81.4
8.8	52.6	21.1	5.8	3.5	0.8	83.8
12.0[b]	1.6	37.8	24.9	7.7	0.2	74.2

[a] About 70 mL of aqueous solution extracted with 50 mL of ethyl acetate-methanol (9:1) each time by agitating for 2 min. [b] pH of the aqueous phase was 6.9 after extraction.

human consumption, was free of residues. Comparable results have been obtained in this laboratory with two other carbamate insecticides of similar structure, methomyl, *S*-methyl *N*-[(methylcarbamoyl)oxy]thioacetimidate (Kiigemagi et al., 1973), and aldicarb, 2-methyl-2-(methylthio)propanal *O*-(methylcarbamoyl)oxime (Kiigemagi et al., 1982), indicating that these types of compounds are largely destroyed during the recovery of peppermint oil by steam distillation. This is in contrast to chlorinated hydrocarbon insecticides, which are frequently concentrated during the distillation of oil (Starr et al., 1963).

The identity of oxamyl residues in some samples was confirmed by gas chromatography–mass spectrometry. The mass spectra of an oxamyl standard after hydrolysis and trimethylsilyl ether formation and that of a field sample are shown in Figure 2 and 3. The mass number data provided by the GC–MS system for the treated samples corresponded to the mass spectrum of an oxamyl standard that showed prominent peaks at masses 59, 72, 99, 102, 145, 187, 219, and 234.

The reliability of the analytical method was tested by adding known amounts of oxamyl to fresh and spent peppermint hay and to peppermint oil prior to extraction and analysis. The range of fortifications and recoveries is shown in Table II. The data indicate that the recoveries for the hay were generally low but consistent. Greater variation of recoveries was found with the oil, particularly with oils recovered with experimental stills in the laboratory. The use of commerically distilled oils resulted in much more uniform recoveries. It is likely that the more severe conditions of distillation used in the laboratory resulted in oils containing more extraneous material that interferred with the analysis.

The storage stability of oxamyl was studied by adding known amounts of the material in acetone solution to peppermint hay, allowing the solvent to evaporate, and then storing the samples at −10 °C in the same freezer with the field samples. The hay samples were fortified at 1.0 ppm, and the average recoveries after 49 months of storage were 54% by gas chromatography and 63% by radiometry. These values are in the same range as the recoveries obtained with samples fortified immediately before analysis. The range of fortifications for the peppermint oil was from 0.1 to 1.0 ppm, and the recoveries averaged 62% by gas chromatography and 65% by radiometry after 11–34 months of storage at 4 °C.

In the development of an analytical method, it is often difficult to determine the efficiency of recovery of sequential steps in a tentative method by using conventional means of detection. Radioisotopes, however, offer a reliable and sensitive means for checking every step in the procedure without interference from crop materials or reagents. Carbon-14-labeled oxamyl was used to check the

Table V. Unlabeled Oxamyl Found in Fortified Peppermint Hay and Oil Corrected by the Radioisotope Dilution Technique

crop[a]	no. of samples fortified	oxamyl added: unlabeled, μg	oxamyl added: labeled,[b] μg	oxamyl found: total[c] amount, μg	oxamyl found: total[c] recovery, %	oxamyl found: sp act., dpm/μg	oxamyl found: radiometric recovery, %	unlabeled oxamyl found corrected by radioisotope dilution technique: unlabeled, μg	unlabeled oxamyl found corrected by radioisotope dilution technique: average, %	unlabeled oxamyl found corrected by radioisotope dilution technique: SD, %
fresh hay[d]	3	2.0	2.0	2.41	60	6712	65	1.74	87	4.7
fresh hay[d]	3	4.0	2.0	3.48	58	4593	64	3.46	87	9.4
fresh hay[d]	3	6.0	2.0	4.88	61	3331	66	5.53	92	17.0
spent hay[d]	3	2.0	2.0	2.28	57	6902	63	1.63	82	4.0
spent hay[d]	3	4.0	2.0	3.42	57	4920	65	3.10	77	3.7
oil[e]	13	1.0	1.0	1.21	61	6592	63	0.90	90	7.4
oil[e]	3	5.0	1.0	3.96	66	2286	70	4.49	90	7.9
oil[e]	3	10.0	1.0	7.59	69	1153	71	9.88	99	1.4

[a] Hay and oil fortified before extraction. [b] Average specific activity 12544 dpm/μg. [c] As determined by gas chromatography. [d] Twenty-gram sample. [e] Ten-gram sample.

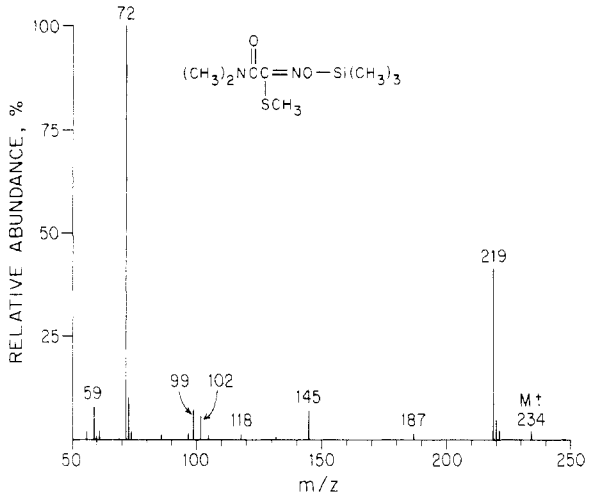


Figure 2. Electron impact mass spectrum of the trimethylsilyl ether of the oxime of oxamyl. Spectrum obtained on a Finnigan 4023 instrument. Ionization voltage was 70 eV.

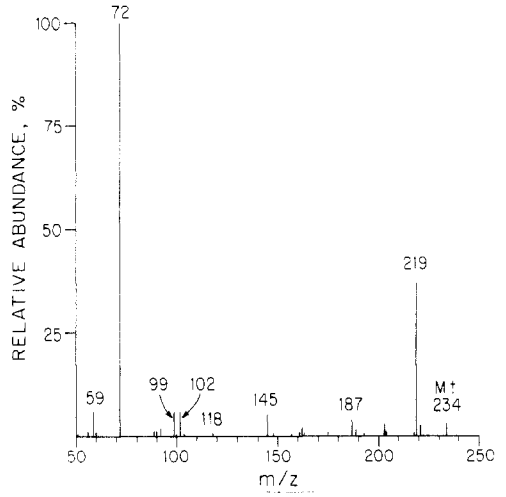


Figure 3. Electron impact mass spectrum of fresh hay sample no. 36; 0.44 of ppm oxamyl. Spectrum obtained on a Finnigan 4023 instrument. Ionization voltage was 70 eV.

individual steps during the development of this method. Results in Table III indicate that losses occurred during the extraction of the oxime from the hydrolysis mixture, during silica gel chromatography, and during concentration of the final solution for derivative formation and gas chromatography. Several solvents and solvent mixtures were tested for the extraction of oxime including ethyl acetate, chloroform, diethyl ether, benzene, chlorobenzene, toluene, and mixtures of ethyl acetate–methanol, ethyl acetate–ethanol and chloroform–diethyl ether–ethanol. Best results were obtained with a 9:1 mixture of ethyl acetate–methanol. The influence of pH on the extraction of the oxime into the solvent was also studied. The results in Table IV indicate that the pH of the aqueous phase does not greatly influence the partitioning of the oxime into the solvent with the exception of highly alkaline pH.

Because of the low recoveries obtained with the gas chromatographic method, the radioisotope dilution technique was used to correct the results. The radioisotope dilution technique depends on the fact that if a radioactive tracer is mixed with the corresponding unlabeled compound, the amount of activity per unit of the substance will be reduced; i.e., the radioactive material will be diluted with the inactive material. If the reduction of activity per unit can be measured, then the amount of diluting material can be calculated. Since the [^{14}C]oxamyl was added to each sample before extractions and the radioactivity was measured when the analysis was completed, the recovery of oxamyl in each individual sample could be calculated and the results corrected. To validate this technique, samples of peppermint hay and oil were fortified with known amounts of labeled and unlabeled oxamyl, followed by extraction and analysis. The recoveries of the unlabeled material were then corrected by the radioisotope dilution technique. Data in Table V show acceptable recoveries after correction. The advantage of using a radioisotope dilution technique is that it provides an independent means for correcting for low or irregular recoveries. The method compensates for losses incurred because of volatility, adsorption on plant tissues, or incomplete solvent extraction, and because the results for each individual sample can be corrected, the data provided by using this technique are more reliable and accurate than the data produced by other residue methods. In fact, the usual procedure for calculating recoveries is to fortify separate samples with the chemical being analyzed and assume that the recoveries between fortified and actual samples will be similar. The radioisotope dilution method provides an internal check on recovery. Another advantage of this method is that accidental loss can be corrected and a valid, accurate residue analysis can be carried out even if only a small fraction of the sample is available.

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LITERATURE CITED

Åkerblom, M. *Pestic. Sci.* **1974**, *5*, 517.
Angoso, M. M.; De Osso, J.; Grau, A.; Dominguez, G. *J. Radioanal. Chem.* **1973**, *13*, 149.
Chapman, R. A.; Harris, C. R. *J. Chromatogr.* **1979**, *171*, 249.
Chiba, M.; Veres, D. F.; Townshend, J. L.; Potter, J. W. *J. Agric. Food Chem.* **1983**, *31*, 53.
Davis, P. L.; O'Bannon, J. H.; Munroe, K. A. *J. Agric. Food Chem.* **1978**, *26*, 777.
Greenberg, R. S. *J. Assoc. Off. Anal. Chem.* **1981**, *64*, 1216.
Holt, R. F.; Pease, H. L. *J. Agric. Food Chem.* **1976**, *24*, 263.
Kiigemagi, U.; Thomson, P. A.; Tinsley, J.; Deinzer, M. L., Oregon State University, Corvallis, OR, unpublished data, 1982.
Kiigemagi, U.; Wellman, D.; Cooley, E. J.; Terriere, L. C. *Pestic. Sci.* **1973**, *4*, 89.
Kohli, K. K.; Albro, P. W.; McKinney, J. D. *J. Anal. Toxicol.* **1979**, *3*, 125.
Redeman, C. T.; Meikle, R. W. *Adv. Pest Control Res.* **1958**, *2*, 183.
Singhal, J. P.; Khan, S.; Bansal, O. P. *J. Agric. Food Chem.* **1977**, *25*, 377.
Starr, H.; Kiigemagi, U.; Terriere, L. C. *J. Agric. Food Chem.* **1963**, *11*, 482.
Thean, J. E.; Fong, W. G.; Lorenz, D. R.; Stephens, T. L. *J. Assoc. Off. Anal. Chem.* **1978**, *61*, 15.

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Evaluation of Cotton Polyphenols as Factors of Resistance to Root-Knot Nematode and Fusarium Wilt

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The terpenoid aldehyde content of cotton (*Gossypium hirsutum* L.) roots of a root-knot nematode (*Meloidogyne incognita* Chitwood and Otiefa) resistant strain (A623) was higher initially and increased faster after inoculation than that of a susceptible strain (M-8). The presence of gossypol and five other terpenoid aldehydes in root exudates was confirmed. These terpenoid aldehydes were also found in the soil surrounding roots where cotton plants had grown; highest concentrations occurred in soils around the resistant inoculated strain. There was not a significant negative correlation of terpenoid aldehyde concentrations in healthy roots with root-knot nematode egg masses in infected roots of 10 other strains of cotton. However, the terpenoid aldehyde concentrations of roots of 17 cotton strains were significantly negatively correlated with fusarium (*Fusarium oxysporum* f. *vasinfectum* (Atk.) Snyd. & Hans) wilt incidence.

Root-knot nematode (*Meloidogyne incognita* Chitwood and Oteifa) and fusarium wilt caused by *Fusarium oxysporium* f. *vasinfectum* (Atk.) Snyd. & Hans (FOV) can significantly limit cotton (*Gossypium hirsutum* L.) production (Smith, 1953). The root-knot nematode (RKN) alone retards plant growth by attacking the root system, causing galling of the roots and initiating other debilitating effects. The incidence and severity of many diseases of seedlings and fusarium wilt (FW) of mature plants are increased in the presence of the nematode (Cauquil and Shepherd, 1970; Martin et al., 1956).

Since 1965, work to breed cotton genotypes resistant to this nematode has resulted in the development and release of Auburn 623 RNR and the identification of several other promising strains that are now being evaluated (Shepherd, 1974, 1979a,b, 1982). These strains typically limit root-knot nematode reproduction to less than 1000 eggs per plant in 40 days following inoculation of seedling plants with 8000 eggs per plant. Progress in incorporating nematode resistance into cotton has been slow largely because identifying resistant plants in segregating populations is a laborious process. The need is evident for a rapid chemical screening procedure.

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A number of secondary plant constituents in cotton have been attributed to impart resistance to pests. Those constituents include gossypol and a number of gossypol-related triterpenoids, sesquiterpenoid quinones, hemigossypols, and heliocides (Bell and Stipanovic 1977), flavonoids (Hedin et al., 1968), an anthocyanin (Hedin et al., 1967), and condensed tannin (Chan et al., 1978). Veech (1978) reported that RKN infection induced synthesis in cotton roots of some methoxy-substituted terpenoid aldehydes, methoxyhemigossypol, methoxygossypol, and dimethoxygossypol. Veech (1979), in a continuation of this study, investigated the histochemical localization and nemotoxicity of the terpenoid aldehydes in cotton. The terpenoid aldehydes were toxic to nematodes, and they accumulated most rapidly and intensely around the head of the nematode in the pericycle of resistant cultivars. Mace et al. (1974) reported on the histochemistry and isolation of gossypol and related terpenoids in healthy roots of cotton seedlings.

Mace and Howell (1974) and Mace et al. (1978) identified the flavonols catechin and gallocatechin and hypothesized that these were condensed proanthocyanidin (tannin) precursors in the roots and stem steles of healthy and verticillium-wilt inoculated cottons. The flavonols increased upon infection. Howell et al. (1976) found that the concentrations of catechin, gallocatechin, isoquercitrin, and condensed tannins were higher in *Verticillium dahliae* Kleb. infected resistant cotton leaves than in infected, susceptible leaves.

Bugbee (1970) studied vascular responses of cotton to infection by FOV. Harrison and Beckman (1982) studied