Determination and Identification of *S*-Methyl *N*-[(Methylcarbamoyl)oxy]thioacetimidate (Methomyl) Residues in Tobacco

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A method for the determination of methomyl (*S*-methyl *N*-[(methylcarbamoyl)oxy]thioacetimidate) in tobacco is described. After ethyl acetate extraction of methomyl from the tobacco and cleanup by solvent partitioning, the pesticide was converted to its volatile oxime derivative (methyl *N*-hydroxythioacetimidate) by alkaline hydrolysis. Subsequent gas chromatographic determination of the oxime derivative was carried out in a gas–liquid chromatograph (GLC) equipped with a flame photometric detector (FPD) and a 394-nm sulfur filter. The method was used to analyze both green and cured tobacco with a sensitivity of about 0.01 ppm based on a 50-g sample. The average recovery for both forms of tobacco was 87%. Methomyl residues analyzed in the GLC were confirmed by gas chromatography–mass spectrometry (GC–MS), and further identification was provided by thin-layer chromatography (TLC).

Pease and Kirkland (1968) described an analytical method for methomyl based on hydrolysis of the compound to its oxime derivative. The final determinative step was carried out using a microcoulometric gas–liquid chromatograph (GLC) with temperature programming. Reeves and Woodham (1974) criticized the method as erratic, low in sensitivity, requiring large injection volumes, and time consuming when compared to their method of determining the parent methomyl pesticide using a flame photometric detector (FPD) gas chromatograph operated isothermally. However, the extraction and cleanup procedures of Pease and Kirkland (1968) were simpler and produced better recoveries than those described by Reeves and Woodham (1974). Moreover, the oxime derivative of methomyl was found to be more sensitive to analysis by GLC than the parent compound, and operation of the gas chromatograph with temperature programming, although time consuming, gave better resolutions.

Previous publications on the analysis of methomyl by GLC (Pease and Kirkland, 1968; Williams, 1972; Reeves and Woodham, 1974) did not provide identification of the compound. Confirmation of pesticides in all residue work is extremely important owing to the possibility of interfering peaks that might have identical retention times as the pesticides.

The analytical method used in this study is an adaptation of the extraction, cleanup, and hydrolysis procedures of Pease and Kirkland (1968). A FPD gas chromatograph operated with temperature programming was used in the final determinative step, and this provided a simpler and more reliable method for the analysis of methomyl. Identification of the "apparent" methomyl appearing in the gas chromatogram was achieved using gas chromatography–mass spectroscopy (GC–MS). Further identification of the pesticide was obtained by thin-layer chromatography (TLC).

EXPERIMENTAL SECTION AND RESULTS

Apparatus and Reagents. The gas chromatograph used was a Tracor MT-220 model equipped with an FPD and a 394-nm sulfur filter. The chromatographic column used was a 1.7 m long U-shaped glass tubing, 5 mm o.d., 4 mm i.d., containing 4.5% Carbowax 20M TPA on 70–80 mesh Chromosorb G which had been acid washed and treated with dimethyldichlorosilane (Varian Aerograph, Walnut Creek, Calif.).

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The mass spectrometer used was a Joel JMS-D100 model. The GC–MS equipment employed was a Pye Series 104 gas chromatograph–magnetic sector single focusing mass spectrometer (constructed in the Department of Physical Chemistry, La Trobe University, Bundoora, Victoria, Australia) coupled to a PDP-CDC 9 computer system.

The reference sample of the oxime derivative of methomyl (methyl *N*-hydroxythioacetimidate) was obtained from the Agrichemicals Department, Du Pont (Australia) Ltd. All the solvents used were redistilled AnalaR grade. Triethylamine was obtained from the British Drug Houses (Australia) Ltd.

The 2-phenoxyethanol–silver nitrate chromogenic agent was prepared by dissolving 0.1 g of silver nitrate in 1 ml of distilled water. 2-Phenoxyethanol (20 ml) was added, and the mixture was diluted to 200 ml with acetone. A small drop of hydrogen peroxide (30% w/v) was added. The solution was stored in the dark for 16 hr before use.

TLC adsorbent used was Whatman silica gel (SG 41, Joseph Crosfield and Sons Ltd., England).

Extraction, Cleanup, and Hydrolysis Procedures. Green tobacco of 7 weeks old (50 g) sampled 2 weeks after treatment with methomyl or cured tobacco (50 g) stored for 2 weeks after curing was blended with 250 ml of ethyl acetate in a Waring Blendor at low speed (3500 rpm) for 2 min. The mixture was filtered under suction and the residue blended with a further 250 ml of ethyl acetate and refiltered. Distilled water (50 ml) was added to the combined filtrate and the mixture was transferred to a 1-l. round-bottomed flask. A Vigreux column was placed onto the flask and the mixture was heated on a boiling water bath to remove the ethyl acetate. The remaining aqueous portion was filtered through cotton wool into a 250-ml separating funnel.

The aqueous solution was then acidified with 4–5 ml of sulfuric acid (0.5 *M*) to a pH of approximately 2, and washed with 100-ml lots of hexane until the washings were colorless. The hexane washings were discarded, and the methomyl residues in the aqueous solution were extracted with 3 × 50 ml of chloroform. The combined chloroform extract, in a 250-ml round-bottomed flask fitted with a Vigreux column, was evaporated to about 20 ml on a water bath.

Sodium hydroxide (50 ml, 0.1 *M*) was added to the concentrated chloroform extract, and the residual chloroform was removed on a boiling water bath with occasional stirring. The alkaline solution was heated on the water bath for a further 15 min to ensure complete hydrolysis of methomyl to its oxime derivative, cooled to room temperature, and acidified with 4–5 ml of sulfuric acid (0.5 *M*) to a pH of

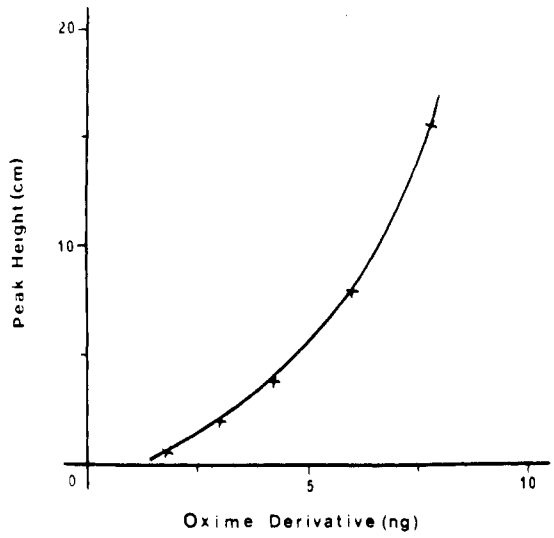

Figure 1. The standard curve of the oxime derivative of methomyl.

approximately 2. The acidified solution was transferred to a 250-ml separating funnel, and the oxime derivative of methomyl was extracted from the solution with 3 × 20 ml of ethyl acetate. The combined extract was dried over anhydrous sodium sulfate and filtered into a 100-ml beaker.

The mixture was concentrated to approximately 20 ml at room temperature under a slow stream of high purity nitrogen after the addition of 0.1 ml of triethylamine. A further 0.1 ml of triethylamine was added to the remaining solution, and the mixture was again evaporated under nitrogen to a small known volume (0.5–5 ml) suitable for analysis by GLC.

Analysis by Gas Chromatography. Temperature programming was used. Initial and final temperatures were 100 and 200°, respectively, and the rate of temperature increase was 15° min^{-1}.

Nitrogen at 67 ml min^{-1} was employed as the carrier gas. The flow rates for oxygen, air, and hydrogen to the FPD were 20, 30, and 150 ml min^{-1}, respectively. The column inlet and the detector temperatures were 215 and 200°, respectively.

The output and input attenuation of 8×10^4 gave a half-scale deflection of the recorder pen with a 9-ng injection of the oxime derivative of methomyl. By way of comparison, a half-scale deflection was obtained with a 27-ng injection of the parent compound. The recorder chart speed was 12 mm min^{-1}.

The GLC column was conditioned by maintaining the oven temperature at 210° with carrier gas flowing at approximately 34 ml min^{-1} for at least 48 hr. Daily conditioning of the column was achieved by introducing two injections each of approximately 120 ng of standard oxime derivative of methomyl prior to the normal operation of the instrument.

A standard curve of the known amount of oxime derivative (nanograms) injected into the GLC against the corresponding peak heights (centimeters) was plotted because of the lack of linearity of the FPD when operated with the sulfur filter (Figure 1). The purified tobacco extracts were injected in the gas chromatograph, and the amount of oxime derivative of methomyl in the injection was determined from the standard curve. Finally, the concentration (parts per million) of methomyl in the extracts was calculated.

Normally, aliquots of 1–5 μl of tobacco extracts equivalent to 50–250 mg of tobacco on a wet weight basis were injected in the gas chromatograph. A retention time of approximately 6.5 min was obtained for the oxime derivative of methomyl utilizing the parameters of operating the GLC already described.

Table I. Residues of Methomyl in 50-g Samples of Green and Cured Tobacco

Type of tobacco	Concn of methomyl, ppm, in Sample 1	Sample 2	Sample 3
Green	1.2	1.2	1.1
Cured	0.04	0.03	0.03

Table II. Recoveries of Methomyl Added to 50-g Sample of Green and Cured Tobacco

Methomyl added to green and cured tobacco in		Recovery of methomyl in Green tobacco in		Cured tobacco in	
μg	ppm	μg	%	μg	%
0.5	0.01	0.41	81	0.4	80
5	0.1	4.4	84	4.1	82
50	1	45	90	46	92
500	10	460	92	470	94
Av % recovery		87		87	

Precision of Determination and Repeatability. The volumes of sample injected were adjusted so that the amount of oxime derivative was within the range of the standard curve (3–7.5 ng). However, whenever possible, the volumes were regulated to contain approximately 4.5–6.5 ng of oxime derivative for more accurate results (see Figure 1). A straight line graph can be obtained for easier determination of the oxime derivative by plotting the standard curve on a log–log graph paper. The limit of detection for methomyl in 50 g of green and cured tobacco on a wet weight basis was 0.01 ppm.

Three lots each of 50 g of green and cured tobacco treated with methomyl were subsampled from two 500-g samples. Analyses of the pesticide were carried out using the method described, and the results are shown in Table I. Reproducible results for both forms of tobacco showed that the method for analysis of methomyl was accurate.

Recovery Experiments. Methomyl was added at four levels, 0.01, 0.1, 1, and 10 ppm, to four 50-g lots of both green and cured tobacco prior to blending, and the samples were analyzed by the above method to determine the efficiency of the whole analytical procedure.

Recoveries of methomyl (0.01–10 ppm) added to green and cured tobacco are given in Table II. Average recovery for both forms of tobacco was 87%, which was 9% more than those reported by Reeves and Woodham (1974), and 6% less than the recovery of Pease and Kirkland (1968).

A 50-g sample of the field-treated green tobacco (7 weeks old) was exhaustively extracted using the procedure of extraction described. The extracts were analyzed separately to determine the completeness of the extraction. Successive extractions gave levels of 0.9, 0.2, 0.04, and 0.01 ppm representing 78, 17, 3, and 0.9% of the total extractable methomyl in the sample, respectively. Thus, two extractions giving a 95% extraction were considered adequate in the procedure. Exhaustive extractions of a 50-g sample of cured tobacco gave similar results.

Identification of Methomyl by GC–MS. Since the Pye gas chromatograph in the GC–MS computer system was different from the selective FPD Tracor gas chromatograph, different conditions for gas chromatography for the analysis of methomyl were required. A separate unit of the

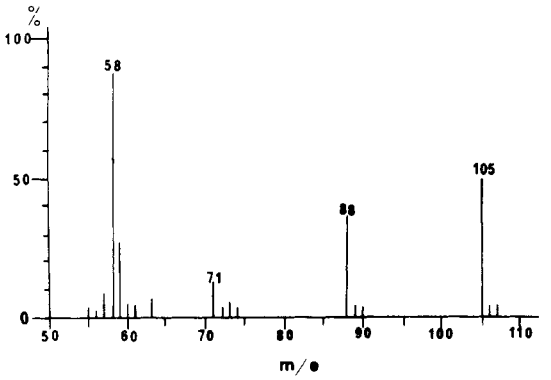

Figure 2. Major features of the mass spectrum of the oxime derivative of methomyl.

Pye instrument identical with the one in the GC–MS system was employed to determine these conditions.

The column used for gas chromatography was a 1.4 m long coiled glass tubing, 3 mm o.d., 1 mm i.d., containing the same packing as the U-shaped column described previously. Nitrogen at 10 ml min^{-1} was used as the carrier gas. The hydrogen and air flow rates to the detector were 30 and 500 ml min^{-1}, respectively. The gas chromatograph was operated with temperature programming under the same conditions as described above in the FPD section. The sensitivity of the instrument was adjusted to obtain half-scale deflection of the recorder pen with a 10-μg injection of the oxime derivative of methomyl. The recorder chart speed was 1 cm min^{-1}. The retention time (9.5 min) of the oxime derivative was noted on the gas chromatogram.

The column was then transferred to the Pye gas chromatograph in the GC–MS computer system. Helium at 10 ml min^{-1} was employed as the carrier gas. The sensitivity of the instrument was adjusted to obtain half-scale deflection of the total ions recorder pen with a 10-μg injection of the oxime derivative.

An injection of 10 μg of standard oxime derivative of methomyl was made into the GC–MS system. The computer was set to record mass spectrometry data between the interval of 9 and 10.5 min from the time of injection. The MS data obtained from the computer were compared with the spectrum of pure oxime derivative obtained from the JMS-D100 mass spectrometer. Similarly, tobacco extracts, which contained the "apparent" oxime derivatives of methomyl, were injected into the GC–MS system, and the oxime derivatives were identified by the mass number obtained from the computer.

The volumes of sample injected and the operating conditions of the GC–MS computer system varied with the amount of oxime derivative of methomyl present in the extracts, and were optimized to obtain the best results.

The mass number data of the oxime derivative provided by the GC–MS computer system corresponded to the mass spectrum of the pure oxime derivative which showed prominent peaks at masses 58, 71, 88, and 105 (Figure 2). These mass numbers were used to confirm the absence of interfering sulfur-containing materials in the determination of the oxime derivative by flame photometric GLC, and the presence of the derivative in tobacco extracts.

Confirmation of Methomyl by TLC. A glass TLC plate (20 cm × 20 cm) with 0.5 mm thick silica gel coating was prepared, activated at 110° for 2 hr, and cleaned by developing it with ethyl acetate for 1 hr.

The oxime derivative of methomyl (20 μg) in 20 μl of ethyl acetate was spotted on the TLC plate, and 100 μl of tobacco extract containing 2 μg of "apparent" oxime derivative was applied adjacent to the standard by using a chromatocharger. A clear space of about 3 cm from one side of the TLC plate was left unused for a subsequent determination of a blank. The plate was then developed with ethyl acetate to a height of 12 cm from the origin. The developed plate was covered, except for the area containing the reference spot, sprayed with 2-phenoxyethanol–silver nitrate solution, and exposed to uv light (wavelength 366 nm) for 15 min. The oxime derivative of methomyl appeared as a dark brown spot.

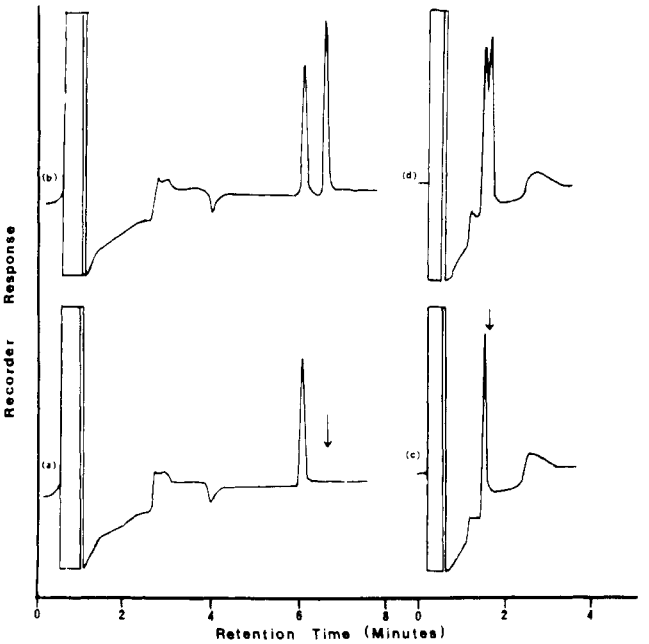

Figure 3. Gas chromatograms of (a) untreated green tobacco analyzed with temperature programming, (b) untreated green tobacco fortified with 9 μg of oxime derivative of methomyl analyzed with temperature programming, (c) untreated green tobacco analyzed isothermally at 180°, and (d) untreated green tobacco fortified with 9 μg of the oxime derivative of methomyl analyzed isothermally at 180°.

The section of silica gel which was adjacent to the reference spot from the unsprayed part of the TLC plate was quantitatively removed as was the unused section of the plate. Both samples of silica gel were extracted five times with 5-ml lots of ethyl acetate. The extracts were concentrated to suitable volumes for analysis by GLC using a slow stream of high purity nitrogen at room temperature.

The uv irradiated spot of oxime derivative had R_f 0.45. There were no significant peaks in the chromatogram of the blank silica gel extract. However, analysis by GLC gave a peak which corresponded to 1.7 ng of oxime derivative in the other silica gel extract. This showed a recovery of 85% from the tobacco extract originally applied on the TLC plate, and hence confirmed the identity of the oxime derivative of methomyl.

DISCUSSION

Although the method outlined above is an adaptation of that of Pease and Kirkland (1968) the modifications and alterations reported in this paper result in considerable improvement. The main alterations are in the method of evaporating ethyl acetate and chloroform extracts and in the final determinative step by GLC. These result in a marked shortening of the procedure without loss of sensitivity or recovery.

Reeves and Woodham (1974) have described the disadvantages of the microcoulometric GLC, and proposed a better method of determination using the FPD gas chromatograph. This improved procedure was used in this study for the final determinative step.

Reeves and Woodham (1974), while attempting to show chromatographic tracings of sediment and water nonfortified and fortified with methomyl, obtained badly resolved double peaks for methomyl in the fortified chromatograms. No satisfactory explanations or remedies were given. However, in the method described here, better resolution was obtained in the chromatograms of green tobacco by operating the gas chromatograph with temperature programming as shown in Figure 3. Similar chromatograms were obtained for cured tobacco.

The oxime derivative of methomyl was analyzed instead of methomyl because the derivative was found to be three times as sensitive as the parent compound under identical conditions of the GLC.

Hence, by combining the two methods, a rapid, sensitive, reliable method of analysis resulting in a high percent recovery of methomyl is developed. The methomyl extracted was also successfully identified as such by GC–MS and TLC.

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LITERATURE CITED

Pease, H. L., Kirkland, J. J., *J. Agric. Food Chem.* **16**, 554 (1968).
Reeves, R. G., Woodham, D. W., *J. Agric. Food Chem.* **22**, 76 (1974).
Williams, I. H., *Pestic. Sci.* **3**, 179 (1972).

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A Survey of the Volatile Constituents of Cotton Lint and Waste with Regard to Byssinosis

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The volatile constituents of lint cotton and gin waste were compared with those of growing buds, bolls, bracts, and hulls by GLC–MS as part of a study on byssinosis, the respiratory disease of cotton mill workers. Of 158 compounds identified from the 5 samples, the growing tissues contained mostly terpenoids, while the lint and waste which have been implicated in byssinosis contained aromatic and alicyclic carbonyl compounds, aromatic alcohols, phenols, esters, lactones, pyrans, epoxides, and pyrazines in addition to the terpenoids. These latter components are believed to be produced in part by microbiological action, oxidation, and heat.

Byssinosis is an occupational respiratory disease which may be caused by inhalation of the dusts of fiber crops such as flax, soft hemp, and cotton. It is characterized by Monday symptoms of chest tightness, cough, and dyspnea accompanied by a marked decrease in expiratory flow rate, and has been associated with an increased prevalence of chronic bronchitis and diminished ventilatory capacity. The prevalence of byssinosis in selected United States textile mills, as determined in five recent independent surveys involving more than 4000 workers, has varied from 20 to 40% in the high-risk preparation areas, and as high as 25% in lower risk yarn processing weaving areas (Merchant et al., 1973).

Byssinosis was first described in Italy in 1705 by Ramazzini, who observed that flax workers developed characteristic respiratory symptoms. Subsequently, investigators such as Prausnitz (1936) and Schilling (1955) described similar respiratory symptoms in cotton-mill workers. At present, the specific causative agents of this respiratory disease are unknown, although Taylor et al. (1971) implicated plant pigments of the flavonoid type, and Tuma et al. (1973) speculated that a bacterial enzyme present in cotton dust might be the causative agent. Model compounds such as methyl piperonylate (Hitchcock et al., 1973) and polymers of quercetin and quercitrin (Kilburn et al., 1973; Hamilton et al., 1973) have been reported to evoke some of the byssinotic symptoms. Steinfeld (1972) reported that phenols and aldehydes were implicated in lung disease.

Similarly, the mechanisms of production of byssinotic symptoms, of decreased flow rates, and of subsequent chronic bronchitis are unknown. Bouhuys et al. (1960) and Bouhuys and Lindell (1961) have suggested that a histamine release in mast cells of the lung occurs. Merchant and associates (1973) described byssinosis as a dose–toxicity–response phenomenon. Although clinical investigators have used human subjects to evaluate samples and fractions, efforts are in progress to develop small-animal bioassays. Kilburn (1972) has exposed hamsters and guinea pigs to respirable dust and subsequently observed recruitment of polymorphonuclear leukocytes through the epithelium of the airways. Changes were also observed in the alveoli, with an increase in macrophages, which had phagocytized the dust particles.

Recently, Merchant and associates (1973) reported that steaming cotton effects a decrease in the byssinotic response by susceptible workers. Almost no drop occurred in the forced expiratory (FEV_1) volume, and only 12% of these workers showed byssinotic symptoms. Although several approaches to a commercially feasible procedure for steaming cotton are being investigated, Merchant and associates suggest that this could best be accomplished at the cotton gin. Later work by Merchant et al. (1974) suggests that steaming reduces dust levels, but does not remove the causative agent.

In 1971, a study was initiated at the Boll Weevil Research Laboratory to survey the chemical constituents of

Boll Weevil Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Mississippi State, Mississippi 39762.