Gas Chromatographic Analysis of Methomyl Residues in Soil, Sediment, Water, and Tobacco Utilizing the Flame Photometric Detector

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An improved method for the gas chromatographic analysis of methomyl in soil, sediment, water, and tobacco is described. Methomyl is extracted from soil, sediment and water with dichloromethane and from tobacco with a mixture of 97.5% dichloromethane and 2.5% benzene. Soil, sediment and water extracts are further purified by elution through a chromatographic column of activated Florisil, while tobacco extracts are purified utilizing a coagulation procedure. The purified concentrated extracts are analyzed on a gas–liquid chromatograph (glc) equipped with a 394-mμ sulfur interference filter. Residues as low as 0.05 ppm may be detected in tobacco, soil, sediment and water samples. The procedure is simple, reliable, and rapid for environmental samples of this type.

In 1971 the USDA's Plant Protection and Quarantine Program initiated a study to monitor residues of the various insecticides utilized to control tobacco pests in North Carolina. Methomyl, *S*-methyl-*N*-[(methylcarbamoyl)-oxy]thioacetimidate, registered as Lannate (formerly Du Pont Insecticide 1179, E. I. du Pont de Nemours & Co.), is one of the more common insecticides used in this area. Although there is no evidence of concentration of this insecticide in the environment, such widespread use required that a simple, reliable method of analysis be available for monitoring any possible accumulation.

Previous analytical procedures (Pease and Kirkland, 1968; Williams, 1972) employ the microcoulometric detector operated in the temperature-programmed mode. Pease (1969) describes a procedure utilizing the flame photometric detector, also operated in the programmed mode. The microcoulometric detection system is erratic, low in sensitivity, requires large injection volumes, is time consuming, and requires reconditioning of the gc column following five to six analyses. The flame photometric detection procedure was a definite improvement but was still time consuming in the programmed mode. All of these methods involve the hydrolysis of methomyl to the corresponding oxime, which also contributed other interfering compounds in the hydrolysis of plant materials.

An improved gas chromatographic prcedure for the analysis of methomyl in soil, sediment, water, and tobacco has been developed. The insecticide is extracted from soil, sediment, and water with dichloromethane and from tobacco with a mixture of 96.5% dichloromethane and 2.5% benzene (v/v). Further purification is accomplished by eluting soil, sediment, and water extracts through activated 60–100 mesh Florisil chromatographic columns and a coagulation procedure for the tobacco extracts; the insecticide residues are then quantitatively measured by flame photometric gas chromatography.

The procedure was designed to also extract and detect methomyl sulfone and sulfoxide, even though other researchers (Harvey and Pease, 1971; Harvey and Reiser, 1971) have demonstrated that these compounds are not products of methomyl metabolism and are relatively unstable. No indications of these or other metabolites were detected utilizing identical gc operating conditions, as described for the methomyl.

EXPERIMENTAL SECTION

Apparatus and Reagents. The gas chromatograph used was a Model MT-220, Tracor, Inc., Austin, Tex., equipped with a flame photometric detector (FPD) utilizing a 394-mμ sulfur interference filter. The detector was operated according to the manufacturer's instructions. The chromatographic column was 6-ft long glass tubing, ¼ in. o.d. (3/16 in. i.d.), containing 10% DC-200 on 80–100 mesh Chromosorb W HP (Applied Science Laboratories, Inc., State College, Pa.). Reference samples of methomyl were obtained from the Industrial and Biochemical Sales Division, E. I. du Pont de Nemours & Co., Wilmington, Del. The solvents used were Nanograde, Mallinckrodt Chemical Works, St. Louis, Mo., all chemicals were reagent, ACS grade. The Florisil adsorbent was obtained from the Floridin Co., Pittsburgh, Pa. Chromatographic cleanup columns were glass tubes, 680-mm long × 10 mm i.d. with a Teflon 2A stopcock on the bottom and 125-ml reservoir at the top of the column.

Extraction. Finely chopped representative 50-g tobacco samples were accurately weighed into half-gallon Mason jars, 500 ml of a solvent mixture containing 80.0% dichloromethane and 20.0% acetone (v/v) was added, and the jars were sealed tightly with screw caps and Teflon liners and rotated on a concentric rotator for 4 hr. The 2.5% benzene was added to the dichloromethane to improve extraction efficiency in comparison to dichloromethane alone. The solvent was filtered through glass wool into graduated cylinders, collecting 300-ml aliquots. The aliquots were stored under refrigeration (30°) in sealed amber bottles pending the subsequent cleanup step.

Tests were made to evaluate the extraction of methomyl from tobacco utilizing a maceration procedure, followed by concentric rotation of half-gallon jars compared to the previously described procedure. No significant differences were noted between the two methods of extraction.

Representative 150-g soil samples were weighed into half-gallon Mason jars and 300 ml of dichloromethane was added. The jars were sealed with screw caps and Teflon liners and rotated on a concentric rotator for 4 hr. The solvent was filtered through glass wool into graduated cylinders, collecting 200-ml aliquots. The aliquots were stored in the refrigerator as in the preceding tobacco extraction step. The minute concentrations of water present in the soil did not appear to affect extraction of methomyl from the soil, as indicated in the recovery tests described later. Traces of water in soil increase the extraction efficiency by deactivating the soil, thereby releasing the pesticide from soil particles.

Sediment samples were extracted, aliquoted, and stored utilizing the identical procedure as for the soil, except that 150 g of sodium sulfate was added to each sample to adsorb water.

Moisture content was determined on soil and sediment by heating 100-g samples for 24 hr *ca.* 120°. All residues were corrected for moisture content.

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Representative 300-g water samples were accurately weighed into 1-l. separatory funnels, 100 ml of dichloromethane was added, and the funnels were shaken vigorously. The layers were allowed to completely separate and the lower dichloromethane layer was drained through a layer of sodium sulfate into amber bottles. The aqueous layers were extracted two additional times with fresh 100-ml portions of dichloromethane, draining the extracts through the sodium sulfate into the sample bottles. The bottles were then sealed and stored in the refrigerator, as in the previous extractions.

A series of exhaustive extractions was also conducted to determine extraction efficiency. This was accomplished by extracting the sample material as described previously, filtering, and collecting all available solvent extracts, and then reextracting the material two additional times. The three extracts were purified by Florisil column cleanup and analyzed for methomyl separately by glc. Essentially all of the methomyl was detected in the first extraction, therefore the previously described extraction procedure was utilized.

Cleanup. *Tobacco.* Two-hundred-and-fifty-milliliter aliquots (25 g) from the preceding extraction step were transferred to 250-ml Erlenmeyer flasks and 1.0 ml of a 0.10% Nujol-in-hexane solution was added. The solvent was then evaporated to *ca.* 3 ml in a warm (30–40°) water bath with a gentle stream of dry air. Five milliliters of acetone and 40 ml of a coagulating solution (1.25 g of ammonium chloride and 2.5 ml of phosphoric acid diluted to 1 l. with distilled water) were added and the solution was warmed in a hot (*ca.* 90°) water bath for 2 min and then allowed to cool for 30 min. The solution was filtered through a Celite (*ca.* 3/8 in.)-glass wool filter into 250-ml separatory funnels, the flasks were rinsed twice with fresh 25-ml portions of the coagulating solution, followed by a rinse with 5 ml of acetone, filtering each rinsing through the Celite filters into the separatory funnels. The aqueous solutions were extracted with 50 ml of dichloromethane, and then twice with fresh 25-ml portions of dichloromethane. All dichloromethane rinsings were drained through anhydrous sodium sulfate to remove water, collecting the solvent in 125-ml flasks. One milliliter of 0.5% OV-210 gc stationary phase (Applied Science) in acetone was added and the solvent evaporated to *ca.* 0.25 ml in a warm (30–40°) water bath with a gentle stream of dry air. Residues were quantitatively transferred to 15-ml glass-stoppered centrifuge tubes. Solvent volumes were adjusted to 10 ml with benzene; the purified extracts were stored under refrigeration pending subsequent glc analysis.

Although the tobacco extracts were purified by utilizing the coagulation procedure, which removed most of the fats, waxes, and oils, some tobacco and other crop extracts may still require the Florisil chromatographic column cleanup described for soil, sediment, and water. In certain instances, soil, sediment, and water extracts may not require any cleanup; this must be determined by the individual analyst and laboratory, depending on the maturity of the crop material, type of crop material, type of soil, sediment, or water analyzed and many other factors. The procedure reported herein pertains to samples analyzed by this laboratory and may vary with the exceptions listed above.

Soil, Sediment, and Water. The aliquots from the extraction step were transferred to 500-ml Erlenmeyer flasks, 1.0 ml of a 0.10% Nujol-in-hexane solution was added, and the solvent was evaporated to *ca.* 10 ml in a warm (40–50°) water bath with a gentle stream of dry air. The concentrated extracts were transferred to chromatographic columns containing 5 g of activated (4 hr *ca.* 120°) Florisil prewet with 15 ml of dichloromethane and allowed to elute dropwise, discarding these eluates. The columns were then eluted with 75 ml of a 20% diethyl ether–80% dichloromethane solution which was also discarded. Finally the methomyl was eluted from the adsorbent with 100 ml of a 90% dichloromethane–10% (v/v) acetone solution and collected in 250-ml Erlenmeyer flasks. One milliliter of a 0.10% Nujol-in-hexane solution was added and the solvent evaporated to dryness in a warm (40–50°) water bath with a gentle stream of dry air. The residues were redissolved in benzene and quantitatively transferred to stoppered 15-ml centrifuge tubes. The tubes were stoppered and stored in a refrigerator pending subsequent glc analysis. All samples were analyzed within 2 days following cleanup. Tests were not made to determine the length of time methomyl extracts may be stored before decomposition begins.

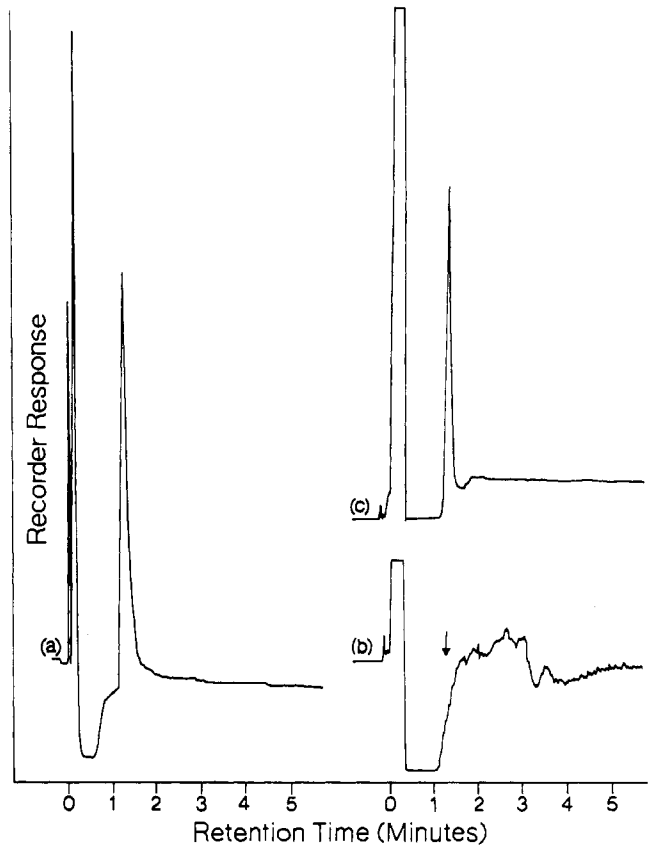

Figure 1. Gas chromatograms of (a) methomyl standard (10.61 ng), (b) untreated tobacco, and (c) untreated tobacco fortified with 2.00 ppm of methomyl. Instrument operating parameters are given in the text.

A series of controls consisting of a solvent check, blank sample, and fortified sample was prepared, then extracted and cleaned up as described previously. These checks were necessary in order to determine if the solvents, glassware, or adsorbent were contaminated, if appreciable interference was present in untreated material, and to determine extraction efficiency of the procedure.

Gas Chromatographic Analysis. The gas chromatograph was operated isothermally as follows. Column, inlet, and detector temperatures were 140, 250, and 210°, respectively. Flow rates were 75, 20, 40, and 80 ml/min for the hydrogen, oxygen, air, and nitrogen, respectively. The hydrogen, oxygen, and air flow rates for this detector were very critical. Operating conditions will vary with different instruments and must be optimized to attain the best results. Sensitivity was adjusted to obtain half-scale deflection of the recorder pen with a 20-ng injection of methomyl. Recorder chart speed was 30 in./hr.

The glc column was conditioned by maintaining the oven temperature at 200° with nitrogen flowing through it for 48 hr. Injections of a concentrated methomyl standard

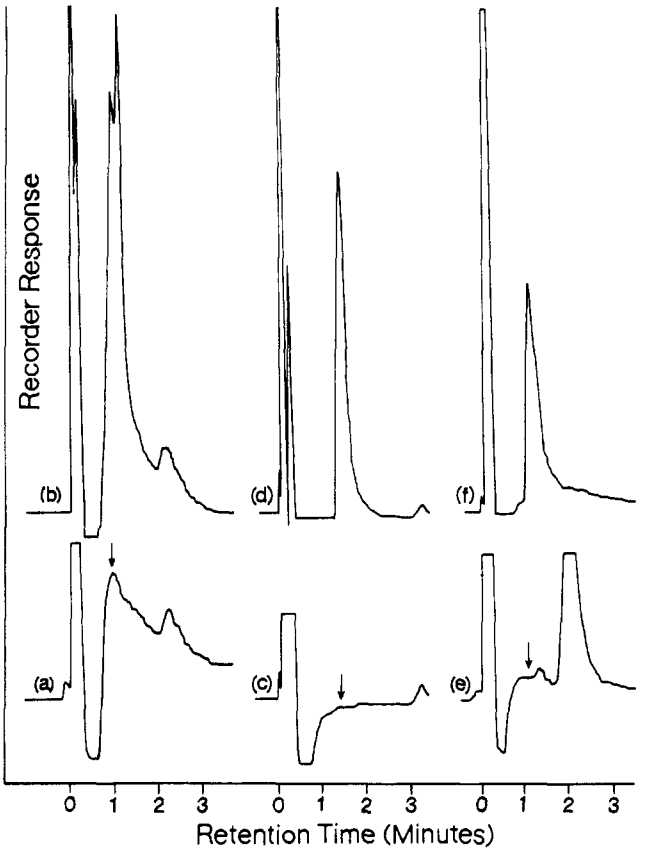

Figure 2. Gas chromatograms of (a) untreated sediment, (b) untreated sediment fortified with 0.07 ppm of methomyl, (c) untreated soil, (d) untreated soil fortified with 0.07 ppm of methomyl, (e) untreated water, and (f) untreated water fortified with 0.03 ppm of methomyl. Instrument operating parameters are given in text.

(*ca.* 100 ng/injection) were made at hourly intervals to further equilibrate the column.

Injections of the purified extracts (normally 2–5 μl) were made on the gas chromatograph utilizing instrument operating parameters previously described. Recorder responses (peak height) of the samples were compared with response obtained with injections of a known concentration of the methomyl standard and ppm of methomyl calculated. Linearity was determined to range between 5.0 and 30.0 ng of methomyl. Precautions were taken to maintain linear conditions at all times due to the narrow range of linearity for the FPD detector when operated in the sulfur mode.

RESULTS AND DISCUSSION

Methomyl was gas chromatographed at residue levels as the parent compound. The applicability of this gas chromatographic method has been demonstrated on samples of tobacco, soil, sediment, and water with reliable results. A retention time of approximately 1 min was obtained for the methomyl with only one small interfering peak detected in an untreated sediment sample. Residue data from these samples will be given in a later report.

Lower limits of detection were determined to be 0.05 ppm for the soil, sediment, and tobacco and 0.01 ppm for water. Average recoveries of 75.1, 90.8, 80.1, and 78.0% were obtained for the water, soil, sediment, and tobacco, respectively. No residues were reported that were not at least twice the noise level.

Chromatographic tracings are shown in Figures 1 and 2 for the methomyl standards, nonfortified, and methomyl fortified soil, sediment, water, and tobacco. Normally aliquots of 2–5 μl were injected on the gas chromatograph, equivalent to 10 mg of tobacco, 25 mg of soil or sediment, and 60 mg of water. Extra peaks were obtained in charts b and f, forming double peaks. These peaks were apparently a result of the lack of linearity on the flame photometric detector for interfering compounds. Sufficient resolution was obtained to accurately identify and quantitate the methomyl peaks.

The highly selective and sensitive flame photometric detector proved to be a valuable tool in the analysis of methomyl in various substrates. Most interfering compounds were eliminated in the extraction or cleanup procedure. With the sensitivity and selectivity of the detector in the sulfur mode, most types of environmental samples may be analyzed for methomyl by this gas chromatographic procedure.

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