About 98% of thiabendazole added to surface strippings and 85% of thiabendazole added to ground fruit can be recovered when fortified between 0.2 and 6.0 ppm (Norman *et al.*, 1972). It was not determined whether the thiabendazole is degraded or is bound in some way by the citrus constituents to prevent extraction from the ground fruit. Until more efficient procedures are developed to extract thiabendazole from ground fruit, residues from the surface and the ground stripped fruit should be combined.

To determine the change in thiabendazole residues on navel oranges during 5 weeks' storage at 42°, three replications of oranges were sampled weekly (Table VI). Surface residues and ground stripped fruit residues were determined. Little difference was noted the first week, but total residue decreased 18, 21, 31, and 38% of the initial value after 2, 3, 4, and 5 weeks, respectively. The surface residue decreased with storage and the ground stripped fruit residue did not increase substantially. Therefore, for highest residue values after storage, residues from the surface and ground stripped fruit should be combined. The stability of thiabendazole on citrus and the effect of constituents normally found in citrus on thiabendazole are not known. Until more information is available, the apparent loss of thiabendazole during storage cannot be explained.

Stability of Citrus-Stripping Solutions. Stripping solutions must be analyzed as soon as possible for highest residue values. Nineteen stripping solutions with an average of 1.86 ppm of thiabendazole decreased during 4 weeks' storage at 50° to an average of 1.32 ppm, a loss of 29%. Cause of this loss has not been determined. Our experience shows that stripping solutions can be kept in a freezer for a few days, but long storage studies have not been carried out.

Interlaboratory Variation. Thiabendazole results from separate samples from the same treatments by three or four different laboratories were compared. The results varied as follows: (test 1) 2.8, 2.5, 2.6, and 3.1; (test 2) 2.1, 1.5, 1.8, and 2.0; (test 3) 5.0, 4.6, 4.3, and 4.7; (test 4) 2.8, 2.3, and 2.3; (test 5) 3.4, 4.0, and 3.4; and (test 6) 9.4, 9.9, and 9.4. The differences among laboratory results are small, since several days lapsed between some analyses, methods varied among laboratories, and samples and sample sizes were not identical. With standardization of methods, adequate sampling, and the combination of surface residues and the residue in the ground stripped fruit, interlaboratory variation should be minimized.

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Received for review June 16, 1972. Accepted August 14, 1972. Mention of a proprietary product in this paper does not constitute a recommendation or an endorsement of the product by the U. S. Department of Agriculture.

Determination of Systemic MBC Residues in Food Crops Treated with Benomyl Fungicide

Earl R. White* and Wendell W. Kilgore

A procedure for the determination of benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazole carbamate] and its decomposition product, MBC (methyl 2-benzimidazole carbamate), on various food crops is described. The compounds are first extracted with benzene and partitioned into 0.1 *N* hydrochloric acid. The acidic layer is washed several times with chloroform and then neutralized to pH 7.8–8.2 with concentrated sodium hydroxide. The single residual product, MBC, is partitioned into ethyl acetate, concentrated by evaporation, and subsequently developed on a commercially prepared thin-layer chromatogram containing a fluorescent indicator. The spot on the developed chromatogram having the same retention time as the standard methyl 2-benzimidazole carbamate is extracted with methanol and measured in an ultraviolet spectrophotometer at 287 mμ. The lower limit of sensitivity for this method is 0.05 ppm. Overall average recovery of benomyl residues obtained from fortified control samples was 87%. Recovery from grown-in labeled MBC residues averaged 83%.

Benlate benomyl fungicide (du Pont) [methyl 1-(butylcarbamoyl)-2-benzimidazole carbamate] has been registered for use in the control of certain diseases of stone fruits (EPA, 1971). The decomposition product, MBC (methyl 2-benzimidazole carbamate), is also a broad spectrum fungicide and is potentially useful at the same low dosage rates as benomyl (Kilgore and White, 1970).

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Current analytical procedures for the determination of benomyl residues are based upon the fluorometric and colorimetric methods developed by Pease and Gardiner (1969). The present paper describes a simple but sensitive thin-layer chromatographic and ultraviolet spectrophotometric procedure designed for the positive identification and quantita-

tive determination of benomyl and MBC residues on a wide variety of food crops.

MATERIALS AND EQUIPMENT

Chemicals. The reference sample of benomyl was supplied by E. I. du Pont de Nemours & Co., Inc., Wilmington, Del. The reference sample of methyl 2-benzimidazole carbamate (SK&F No. 26058) was supplied by Smith Kline & French Laboratories, Philadelphia, Pa. The 2-^{14}C-labeled methyl 2-benzimidazole carbamate was synthesized from ^{14}C-thiourea purchased from New England Nuclear, Boston, Mass.

Spectrophotometers. Infrared spectra were derived from potassium bromide disks, utilizing a Perkin-Elmer Model 337 spectrophotometer. Ultraviolet spectra were acquired through the use of a Hitachi Perkin-Elmer Model 139 spectrophotometer.

Thin-Layer Chromatograms. Precoated plastic sheets (Polyamide II/UV-254, with fluorescent indicator) were purchased from Brinkmann Instruments, Inc., Westbury, N. Y.

Radioactivity Measurements. The radioactivity (^{14}C) present in all stages of the analysis was measured in a Model 314 EX Packard Tri-Carb scintillation counter. The scintillator fluid was composed of 15 g of PPO, 0.3 g of POPOP, 150 g of naphthalene, 1140 ml of dioxane, 720 ml of absolute ethanol, and 1140 ml of toluene.

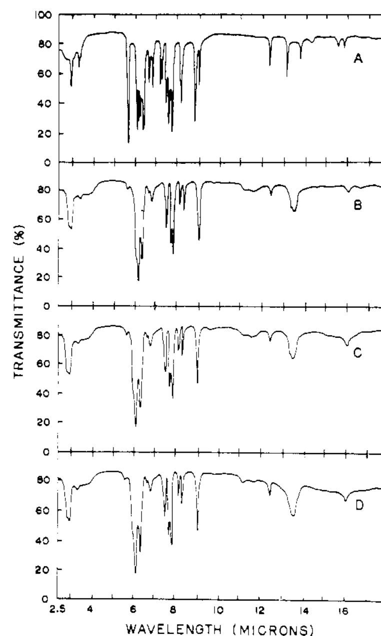

Figure 1. Infrared spectra of (A) benomyl analytical standard, (B) MBC analytical standard (SK&F No. 26058), (C) synthesized 2-^{14}C-MBC, and (D) recovered product of analysis (MBC)

PROCEDURE

Extraction Method. Macerate a representative crop sample (500 g) in a suitable food chopper. Deposit 150 g of the macerated sample into a variable speed blender cup. Add 600 ml of benzene to the macerate and blend the mixture for 5 min. Transfer the blended contents of the cup into a 2-l. Erlenmeyer flask and place the flask on a gyratory shaker for a period of 1 hr. Finally, decant the benzene through anhydrous sodium sulfate and store the extract in a cool dark area until analyzed. For recovery studies, fortify the sample directly with a known amount of benomyl before adding the extracting solvent.

Cleanup and Conversion. Concentrate 400 ml (100 g of crop) of extract to approximately 50 ml by the use of a rotary vacuum concentrator (CaLab, Berkeley, Calif.) at 40°. Transfer the concentrated benzene extract quantitatively into a 250-ml separatory funnel. Add 50 ml of 0.1 *N* hydrochloric acid to the funnel and shake the contents vigorously for 1 min. Cautiously, and frequently, relieve the internal pressure generated within the funnel during the initial partitioning step. Allow the phases to separate and repeat the acid extraction once more, using another 50 ml of 0.1 *N* hydrochloric acid. Collect the combined acid fractions in a 250-ml separatory funnel and discard the benzene layer. Wash the aqueous phase three times with 50-ml portions of chloroform and discard the chloroform after each phase separation. The process may be interrupted at this point, since MBC is relatively stable to further chemical or physical degradation.

Neutralize the acidic aqueous phase to pH 7.0–7.5 with concentrated sodium hydroxide and then adjust the pH to 7.8–8.2 with dilute (1%) sodium hydroxide. Partition the resultant methyl 2-benzimidazole carbamate into three 50-ml portions of ethyl acetate. Discard the neutralized aqueous phase following the final ethyl acetate extraction. Filter the ethyl acetate extracts through anhydrous sodium sulfate and collect the eluates in a 300-ml round-bottomed flask. Wash the sodium sulfate filter cake with an additional 50 ml of ethyl acetate and concentrate the combined ethyl acetate eluates to approximately 5 ml by the use of a rotary vacuum concentrator at 40°. Transfer the concentrate quantitatively into a graduated sedimentation tube and continue the evaporation to a volume of 0.10–0.20 ml with a gentle stream of dry nitrogen.

Thin-Layer Chromatography. Prepare the Polyamide precoated plastic sheets for use by first developing the sheets in a solvent system containing chloroform, ethyl acetate, and acetic acid (190:10:4). This prewash procedure effectively eliminates streaking of MBC. Allow the solvent-saturated thin-layer sheets to dry at room temperature before applying the prepared samples and reference standards of MBC. Apply all or a quantitatively measured portion of the prepared concentrates to the thin-layer sheet as separate small spots (0.5 cm in diameter) approximately 3 cm apart along an imaginary line, 2 cm from the bottom of the chromatogram. Most prepared extracts can be spotted without overloading the plate. Samples containing a high concentration of sugar or oil, however, may necessitate the use of a streak application of the extract to be chromatographed. Prepare a standard solution of MBC (10 mg) in 10 ml of ethyl acetate and apply 10 μl (10 μg) of this standard solution to each thin-layer sheet to serve as a reference. Develop the chromatogram in the same solvent system and locate the spots under ultraviolet light (2537 Å). The analysis may again be interrupted after washing the plastic sheets or after completion of the developed chromatogram.

Ultraviolet Spectroscopy. Extract the spots or bands having the same R_f (0.7) as the MBC standard from the plate with a vacuum-assisted spot collector (Brinkmann Instruments, Inc., Westbury, N. Y.) and elute the compound from the support medium with absolute methanol. Determine the absorbance at 287 mμ, utilizing semimicro quartz cells having 10-cm light paths for residues less than 0.2 ppm and 1 cm quartz cuvettes for higher residues. Prepare the standard MBC solutions from absolute methanol and use absolute methanol in the reference cell of the spectrophotometer.

Synthesis of 2-^{14}C-MBC. The radiolabeled MBC (specific activity 2.83 μCi/mmol) was synthesized according to Loux (1961) and purified by recrystallization from ethyl acetate.

Table I. Recovery of Benomyl from Various Crops

Crop	ppm Added	ppm Found	Recovery, %	Average recovery, %
Apple	0.05	0.04	80	
	0.10	0.08	80	
	0.50	0.45	90	87
	1.00	0.98	98	
Apricot	0.05	0.04	80	
	0.10	0.09	90	
	0.50	0.50	100	91
	1.00	0.92	92	
Cherry	0.05	0.04	80	
	0.10	0.07	70	
	0.50	0.48	96	83
	1.00	0.84	84	
Grape	0.05	0.05	100	
	0.10	0.10	100	
	0.50	0.49	98	100
	1.00	1.04	104	
Nectarine	0.05	0.04	80	
	0.10	0.07	70	
	0.50	0.48	96	80
	1.00	0.74	74	
Peach	0.05	0.04	80	
	0.10	0.08	80	
	0.50	0.54	108	89
	1.00	0.88	88	
Plum	0.05	0.04	80	
	0.10	0.08	80	
	0.50	0.38	76	79
	1.00	0.78	78	
Overall average				87

Table II. Recovery of Grown-In ^{14}C-MBC from Bean Plants

No. of plants analyzed	Combustion analysis, dpm Macerate	Combustion analysis, dpm Extract	Average recovery, %
3	2980	18,420	86.0
3	10,670	49,730	82.3
3	1690	7950	82.4
3	2020	10,130	83.5
Total 12			
Overall average			83.6

Thin-layer chromatography revealed a single component having the same R_f as the corresponding Smith Kline & French reference compound No. 26058. Structural confirmation was verified from infrared spectra (Figure 1).

Preparation of Grown-In 2-^{14}C-MBC Residues. Preparation of grown-in residues was accomplished by growing bean plants (*Phaseolus vulgaris* L. "Tenderbest") in a modified Hoagland nutrient solution (Johnson *et al.*, 1957) containing radiolabeled MBC (0.5 μCi of MBC/20-ml solution). Radiocarbon content was determined by combustion analysis (Krishna and Casida, 1966).

RESULTS AND DISCUSSION

This analytical procedure is based upon the relative ease in which the parent compound, benomyl, degrades to the simpler and more stable compound, MBC. The degradation product is the compound actually being determined in this method. To convert the determined amount of MBC to benomyl in parts per million, the following formula is used.

$$\text{ppm of benomyl} = \frac{(\mu\text{g of MBC})\,(1.52)}{\text{g of sample analyzed}}$$

where 1.52 = the ratio of the molecular weight of benomyl (290) to the molecular weight of degradation product MBC (191).

Aliquots of freshly prepared benomyl solutions were added to fruit-control macerates prior to extraction. The resulting recovery studies (Table I) ensured the quantitative *in vitro* degradation of benomyl and the total extraction of the degradation product. The extraction efficiency of this analytical procedure was tested further by subjecting actual systemic residue samples to analysis (Table II). The overall average recovery of grown-in 2-^{14}C-MBC compared favorably with the overall average recovery of MBC from the spiked samples in Table I.

The ability to form a water-soluble salt of the degradation product is utilized in the cleanup of crop extracts prior to analysis. Dilute hydrochloric acid hydrolyzes any residual benomyl to its natural degradation product, and in turn converts the degradation product to a water-soluble salt. The organic layer containing the greatest amount of crop material is subsequently discarded. Additional washing of the aqueous layer with an organic solvent reduces the amount of interferences still further. Chloroform was the organic solvent of choice, primarily to reduce the number of separatory funnel transfer steps. Any separation problems at the interface can be resolved by physically breaking up the rag with a glass rod or by centrifugation.

Thin-layer chromatography of the prepared extracts must be done in an exacting manner for greatest success and maximum sensitivity. Radiochemical studies utilizing 2-^{14}C-labeled MBC showed that poor separation and streaking occurred when the procedure was altered. Structural confirmation of extracted spots and bands was accomplished, following quantitation in the ultraviolet spectrophotometer, by evaporating the methanol eluates to dryness and obtaining infrared spectra of the residues (Figure 1).

The present study for the determination of benomyl represents an alternative approach to the method developed by Pease and Gardiner (1969). Although extraction information and recovery rates are similar for both procedures, the present method contains some inherent advantages over the Pease and Gardiner procedure. For example, the required laboratory equipment and apparatus are common to most laboratories; the products of analyses are more stable; the sensitivity is greater through a more complete cleanup of samples prior to analysis; and finally, the analysis may be interrupted indefinitely at various stages in the procedure.

ACKNOWLEDGMENT

The authors express their appreciation to Joseph Ogawa, Bill Manji, and Elaine Bose, Department of Plant Pathology, for their valuable assistance and contributions.

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Received for review February 25, 1972. Accepted July 14, 1972.