Table IX. Phenthoate in Soil beneath Field-Washed Trees[a]

Pan position[b]	ppm at days after spraying	
	3	10
0° P	1.1	1.4
0° 1/2	1.5	1.5
90° P	2.7	0.5
90° 1/2	1.5	1.1
45° P	0.4	1.9
45° 1/2	1.9	0.9
Av	1.5	1.2

[a] Sprayed with 7.5 lb of AI/1500 gal per acre, then washed with 3000 gal/acre of water-detergent solution at 3 and 10 days post-application. [b] Pans of soil placed under trees immediately before washing and removed as soon as trees were dry. Positions as shown in Table VIII.

were approximately the same for the various pan positions, showing very uniform spray coverage of the tree. Except for the initial wetting, the soil remained dry as there was no rain throughout the experimental period. The average residue for all the pan positions remained relatively constant and indicates that the phenthoate present is strongly sorbed to the soil and does not undergo extensive degradation. Iwata et al. (1975) found that when phenthoate was added to moist soil at 500 ppm, 50% of the pesticide was degraded within 10 days and over 95% within 30 days. Thus, the field residues would also be expected to degrade rapidly when the soil is wetted by irrigation or rain.

Pans of soil were placed under the field-washed trees just prior to washing at the 3- and 10-day intervals and removed for analysis as soon as the trees had dried to provide an estimate of the amount of insecticide carried into the soil by washing. These data are in Table IX. Approximately the same amount was recovered from the soil at each interval and these levels were about half of those resulting from runoff when the spray was applied.

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A Rapid Spectrophotometric Method for the Simultaneous Determination of Intact Benomyl and Its Degradation Product, Methyl 2-Benzimidazolecarbamate (MBC), in Organic Solvents and Water

Mikio Chiba

A rapid ultraviolet (UV) spectrophotometric method for determining benomyl and its degradation compound, methyl 2-benzimidazolecarbamate (MBC), has been developed. Maximum absorbances of a solution containing benomyl and MBC occur at 294 nm (A) and 286 nm (B), and, from the ratio A/B, the proportion of intact benomyl and MBC in the solution can be obtained from a standard curve. After determining the proportion of benomyl and MBC in organic solvents, *n*-butyl isocyanate (BIC) is added to the same solution to determine the total quantity of benomyl in the solution. With a nonscanning spectrophotometer, absorption of the sample is measured at 294 nm before and after adding BIC, and both intact and total benomyl concentrations are determined. BIC stabilizes benomyl in most common organic solvents except methanol and ethanol.

Benomyl, methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate, is one of the most widely used systemic fungicides for the control of plant diseases, but its mode of action is not yet clearly understood. Because its breakdown product, methyl 2-benzimidazolecarbamate (MBC), is also fungitoxic (Peterson and Edgington, 1969; Clemons and Sisler, 1969), studies to elucidate its mode of action became complicated.

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The greatest problem in elucidating the mode of action has been in the lack of suitable analytical methods for intact benomyl and MBC. Methods available to determine intact benomyl per se to date include a colorimetric method in the milligram range (Miller et al., 1974), a TLC technique (Clemons and Sisler, 1969; Peterson and Edgington, 1969), and a radioactive technique (Upham and Delp, 1973; Baude et al., 1973), but these are rather impractical for routine quantitative analyses at microgram levels. There are many other methods to determine MBC and further degradation compounds, including fluorometric and colorimetric methods (Pease and Gardiner,

1969; Pease and Holt, 1971), a high-speed liquid chromatographic method (Kirkland, 1973), and a UV spectrophotometric method (Steurbaut et al., 1973). It has been practically impossible, therefore, to check the persistence of intact benomyl after application by analyzing the degradation compounds.

Determination of intact benomyl is difficult because it is heat labile and decomposes rapidly in common organic solvents (Chiba and Doornbos, 1974). Recently, Calmon and Sayag (1976a,b) studied the kinetics and mechanisms of conversion of benomyl to MBC and stated that the conversion is due to spontaneous intramolecular catalysis.

This paper reports the role of *n*-butyl isocyanate (BIC), also produced when benomyl degrades to MBC, in controlling the rate of degradation of benomyl in organic solvents. Furthermore, a simple and rapid spectrophotometric method, in which BIC is employed to determine intact benomyl and MBC simultaneously in organic solvents, has been reported.

MATERIALS AND METHODS

Chemicals. *Benomyl.* Recrystallized benomyl, obtained from E. I. duPont de Nemours & Co., Inc., was used as the standard. Benomyl for general experimental use was prepared from Benlate 50% WP.

MBC. Recrystallized MBC used in these studies was obtained from Dr. F. Von Stryk, Agriculture Canada, Research Station, Harrow, Ontario, or was produced by the author from Benlate 50% WP.

Solvents. Methanol, methylene chloride, ethyl acetate, and benzene were pesticide grade from Caledon Laboratories Ltd. Chloroform and 1,4-dioxane were Analar grade from BDH. Absolute ethanol was from Consolidated Alcohols Ltd. *n*-Butyl isocyanate (BIC) and surfactant Tween-20 were practical grade from J. T. Baker Chemical Co.

Instrument. A Beckman DK-2A ratio recording spectrophotometer with hydrogen lamp was used with a near-IR silica cell, 1-cm light path for holding samples.

Spectrophotometric Procedures. A 10-ppm benomyl solution prepared in chloroform at 25 ± 1 °C was scanned in the UV spectrophotometer. Immediately after dissolving the chemical, the solution was placed in a 1-cm silica cell and the spectrum recorded (0-time sample). The spectrum was recorded every 10 min and, subsequently, every 30 min until no further changes could be detected.

Since benomyl is not readily soluble in water it was essential to first disperse it in water by using either 0.1% methanol, or 0.1% or 1.0% Tween-20. An equal amount of methanol was then added to dissolve the suspended benomyl. Methanol was added immediately before scanning to minimize degradation of benomyl by methanol.

Standard Curves for Pure Benomyl and MBC. Standard solutions of benomyl, 2, 4, 6, 8, and 10 ppm, were prepared in chloroform that contained 1000 ppm of BIC. Absorbance was measured at 294 and 286 nm for each solution, and a standard curve was prepared relating concentration and absorbance.

Standard solutions of MBC stoichiometrically equivalent to 2, 4, 6, 8, and 10 ppm of benomyl were prepared in chloroform and the absorbance was measured at 294 and 286 nm. The actual weight of MBC is 65.9% of that of the corresponding weight of benomyl:

$$\frac{\text{MW of MBC}}{\text{MW of benomyl}} = \frac{191.2}{290.3} = 0.659$$

Standard Curves for Solutions Containing Different Proportions of Benomyl and MBC. Solutions of benomyl and MBC in proportions of 100:0, 80:20, 60:40, 40:60, 20:80, and 0:100, respectively, were prepared to a final concentration equivalent to 10 ppm of benomyl. In the 80:20 solution, for example, there is 8 ppm of intact benomyl and the remainder is MBC stoichiometrically equivalent to 2 ppm of benomyl.

After preparing a solution, the absorbance was determined at 294 and 286 nm immediately to minimize degradation of benomyl. Absorbance at 294 nm was plotted against the proportion of the two chemicals in solution (Figure 1). The ratio of absorbance (A) at 294 nm and (B) at 286 nm for each solution was determined. The percentage of intact benomyl in the standard solutions was then plotted against the A/B ratios (Figure 2).

Unknown Samples in Organic Solvents. A sample chloroform solution was scanned in the range of 270–320 nm of the spectrophotometer. The maximum absorbances were measured at 294 (A) and 286 (B) nm and the ratio A/B was calculated. From Figure 2, the proportion of intact benomyl in the solution (P^*) was determined.

The corresponding absorbance (A^*) of a 10-ppm solution was then read at (P^*) from Figure 1. The concentration of solution (X, expressed as benomyl) was then obtained from the following equation (graphic method).

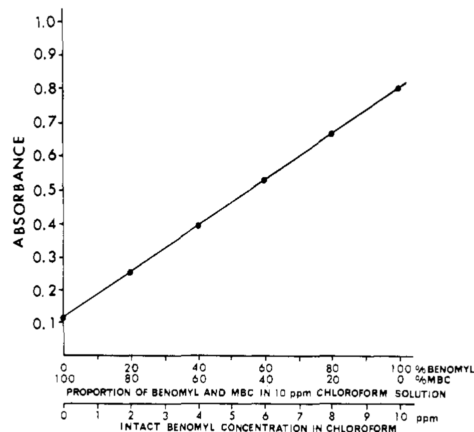

Figure 1. Absorbance at 294 nm of a chloroform solution containing a total of 10 ppm of benomyl and MBC relative to the proportions of benomyl and MBC.

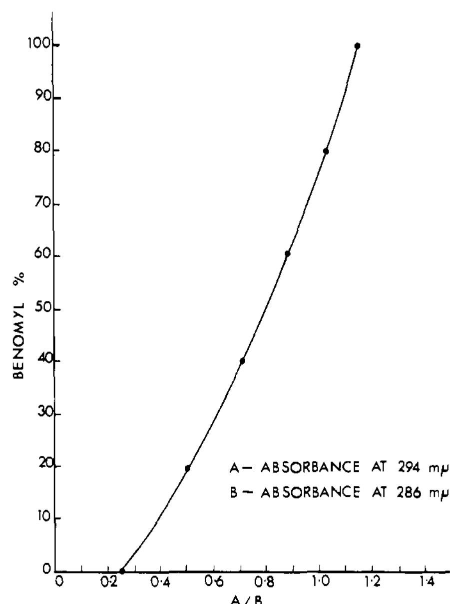

Figure 2. Intact benomyl (percent) in chloroform solutions containing different proportions of benomyl and MBC relative to A/B ratios where A = absorbance at 294 nm and B = absorbance at 286 nm.

$X = 10A/A^*(\text{ppm})$

To 9 portions of the same sample solution, 1 portion of a 100 000-ppm BIC chloroform solution was added and the mixed solution was scanned as before. The maximum absorption at 294 nm (A_{orig}) was checked again and the total benomyl concentration was found from the standard curve for pure benomyl as described in the preceding section on standard curves. The actual concentration of benomyl in the sample solution was obtained by multiplying by 10/9 to correct for the dilution which resulted from adding the BIC solution (BIC method).

Unknown Samples in Aqueous System. Sample suspensions prepared with the aid of methanol (0.1%) and Tween-20 (0.1 and 1.0%), respectively, were mixed with an equal volume of methanol immediately before scanning. The mixed solution was scanned exactly the same way as above and the maximum absorbances at 294 and 286 nm were measured to find the percentage of intact benomyl in the solution. To determine a total concentration of benomyl, 20 ml of the sample suspension was placed in a 60-ml separatory funnel and 10 ml of chloroform was added. The whole mixture was shaken well for 1 min, the chloroform layer was removed, and the chloroform extraction was repeated. The two chloroform extracts were combined, made up to a volume of 20 ml, and analyzed according to the above procedure with BIC.

RESULTS

Spectra of Benomyl, MBC, and Mixtures of the Two Compounds in Organic Solvents and Water. Pure benomyl in chloroform has several absorption peaks in the 230–340-nm range; absorption is strongest at 286 and 294 nm as shown in Figure 3-1. Absorbance varies with the solvents used. For example, the strong absorption peaks noted in chloroform at 254 and 261 nm cannot be observed in benzene because of its opacity below 275 nm. In aqueous suspensions absorbance at 294 nm is not as prominent as it is in organic solvents.

In chloroform the absorption spectrum of benomyl changes gradually with time because it decomposes to MBC which has maximum absorption at 280 and 286 nm. The strong absorption peak at 294 nm (Figure 3-1) when a benomyl solution is prepared becomes less prominent with time and absorption at 286 nm becomes more prominent. The absorption spectrum after 90 min is shown in Figure 3-2. The absorption spectrum continues to change with time (Figure 3-3) and the spectrum stabilizes after 7 h, when only 24% of the benomyl remains intact and the rest has been converted to MBC (Figure 3-4).

The MBC spectrum also varies slightly depending on the solvent used as shown in Figure 4.

Standard Curve for Pure Benomyl and Mixtures of Benomyl and MBC. Standard curves for pure benomyl and for pure MBC in chloroform (up to 10 ppm) are linear through the origin when absorption is read at 286 and 294 nm. With the spectrophotometer used, 10 ppm is the practical upper concentration for either compound.

If benomyl has been dissolved in chloroform for some time, the absorbance at 294 nm is the sum of the absorbance due to intact benomyl and that due to its degradation compound, MBC. Therefore, even if 10 ppm of benomyl completely degrades to MBC there is a certain absorption at 294 nm due to 6.59 ppm of MBC (Figures 4 and 5). Accordingly, the standard curve for benomyl when MBC is present does not go through the origin. The point at which the ordinate is intercepted depends on the

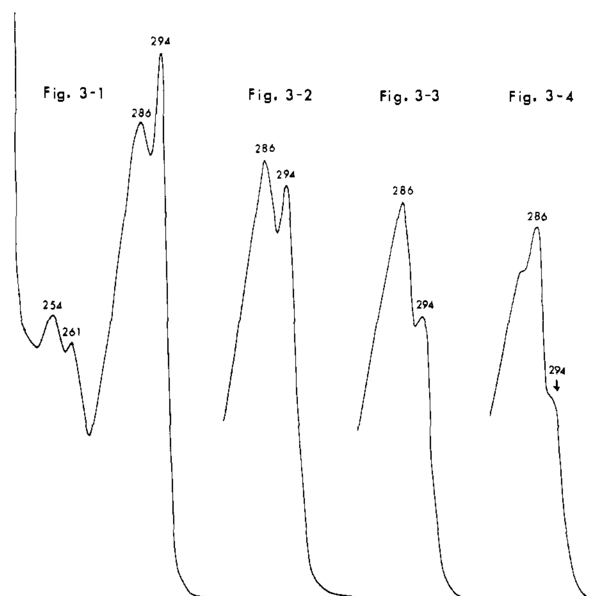

Figure 3. Absorption spectra of 10 ppm of benomyl in chloroform (25 °C) at different intervals after preparing the solution: (3-1) 0 h immediately after preparing the solution; (3-2) 1.5 h; (3-3) 3.5 h; (3-4) 7 h.

amount of MBC. Figure 1 shows the standard curve for benomyl in the presence of different percentages of MBC. Since the solutions were prepared to a constant value of 10 ppm or the equivalent of benomyl, the absorbance reading can be readily converted to the amount of benomyl present and the proportion degraded to MBC.

Proportions of Benomyl and MBC in Unknown Solutions. When benomyl degrades to MBC, absorption intensities at 286 and 294 nm both decrease. The former decreases at a much slower rate, however, because the MBC formed from benomyl also absorbs at 286 nm. Thus, to calculate absorptions at both wavelengths, the following general equation was developed:

$$A = \frac{X_{int}(A_{orig} - A_{MBC})}{X_{orig}} + A_{MBC} \quad (1)$$

where A_{orig} = absorption of X_{orig} parts per million of benomyl at 294 nm, A_{MBC} = absorption of MBC when X_{orig} parts per million of benomyl completely decomposed to MBC at 294 nm, X_{orig} = original concentration (parts per million) of benomyl or total concentration of benomyl after completing the BIC procedure, X_{int} = concentration of intact benomyl at time of analysis, and A = absorption as observed (sum of intact benomyl and MBC absorptions) at 294 nm.

From the values experimentally obtained for A_{orig} (0.803) and A_{MBC} (0.126) when X_{orig} = 10 ppm in chloroform, the following equation is obtained:

$$A = \frac{(0.803 - 0.126)X_{int}}{10} + 0.126$$
$$= 0.0677X_{int} + 0.126$$

Similarly, absorption at 286 nm (B, equivalent to the above A at 294 nm) is obtained as follows:

$$B = \frac{(0.697 - 0.472)X_{int}}{10} + 0.472$$
$$= 0.0225X_{int} + 0.472$$

From the above two equations, the ratios of absorptions at 294 nm (A) and 286 nm (B) were calculated at different

Table I. Absorption Ratios $(A/B)^a$ of Solutions Consisting of Different Proportions of Benomyl and MBC

Composition of system		Solvent									
Benomyl	MBC[b]	Chloroform	Methylene chloride	Ethyl acetate	Benzene	Dioxane	Ethanol	Methanol	0.1% methanol in H_2O^c	0.1% Tween-20 in H_2O^c	1.0% Tween-20 in H_2O^c
100	0	1.153	1.208	1.222	1.232	1.238	1.088	1.151	1.003	1.015	1.089
90	10	1.090	1.141	1.144	1.153	1.156	1.023	1.078	0.955	0.954	1.023
80	20	1.024	1.068	1.061	1.070	1.069	0.955	1.003	0.905	0.893	0.956
70	30	0.953	0.989	0.972	0.980	0.976	0.886	0.924	0.852	0.830	0.886
60	40	0.877	0.904	0.879	0.885	0.877	0.813	0.841	0.796	0.766	0.814
50	50	0.795	0.811	0.776	0.782	0.772	0.738	0.754	0.738	0.700	0.739
40	60	0.706	0.709	0.666	0.672	0.660	0.660	0.663	0.677	0.632	0.662
30	70	0.610	0.598	0.549	0.552	0.539	0.580	0.566	0.613	0.563	0.583
20	80	0.505	0.475	0.422	0.424	0.410	0.496	0.465	0.544	0.493	0.500
10	90	0.391	0.339	0.284	0.284	0.270	0.410	0.358	0.473	0.420	0.415
0	100	0.267	0.187	0.134	0.132	0.120	0.320	0.245	0.396	0.347	0.327

[a] A, absorbance at 294 nm; B, absorbance at 286 nm. [b] Calculated as benomyl. [c] Mixed with equal amount of methanol.

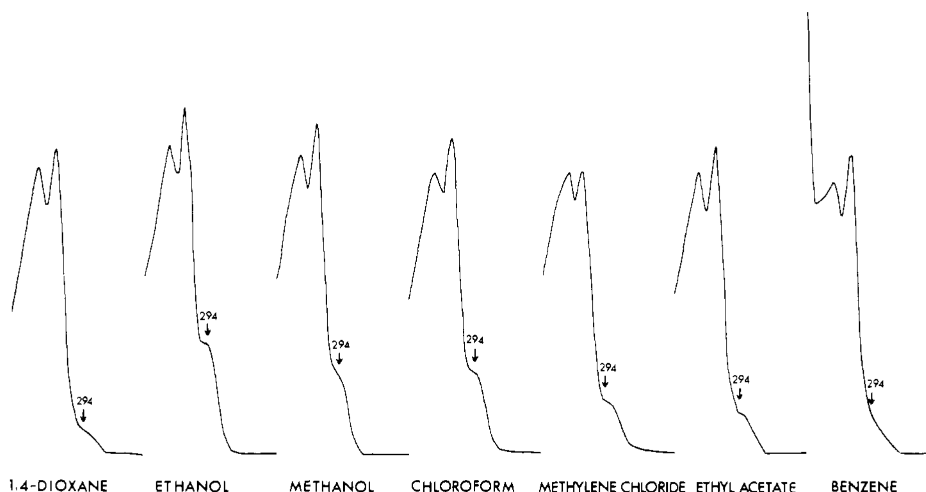

Figure 4. Absorption spectra of MBC in organic solvents (MBC concentration, 6.59 ppm, equivalent to the MBC stoichiometrically produced from 10.0 ppm of benomyl).

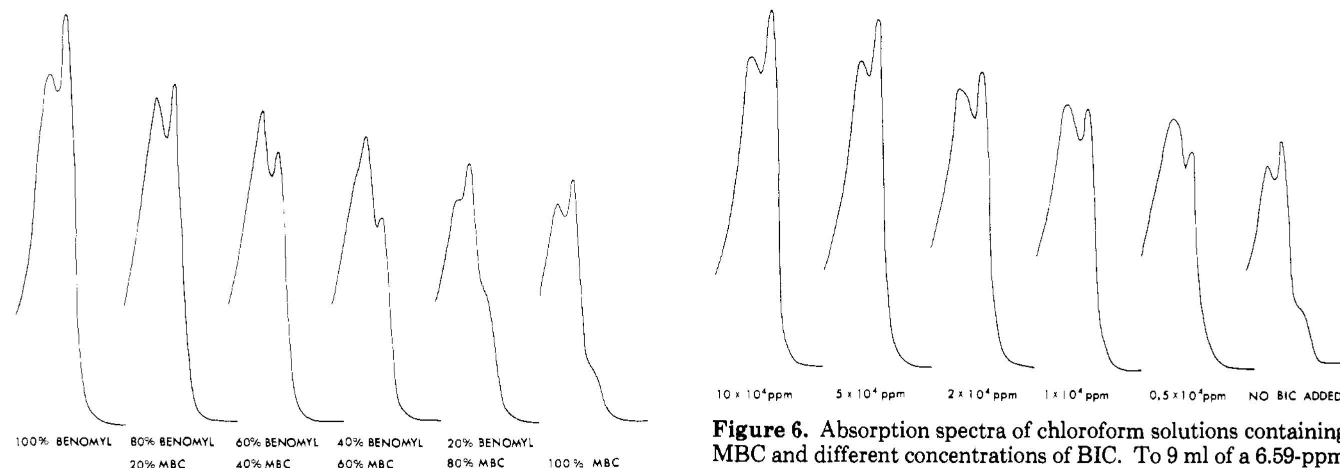

Figure 5. Absorption spectra of chloroform solutions containing different proportions of benomyl and MBC. The total concentration is 10 ppm of benomyl or the equivalent. The percentage of MBC listed is that equivalent to the percentage of benomyl degraded.

X_{int} values with 10% intervals as shown in Table I. Included in Table I are the ratios of A/B with six other solvents and three aqueous systems. When the values obtained in chloroform are plotted, the curve is identical with Figure 2; the proportion of remaining benomyl in chloroform could be identified because the value of A/B

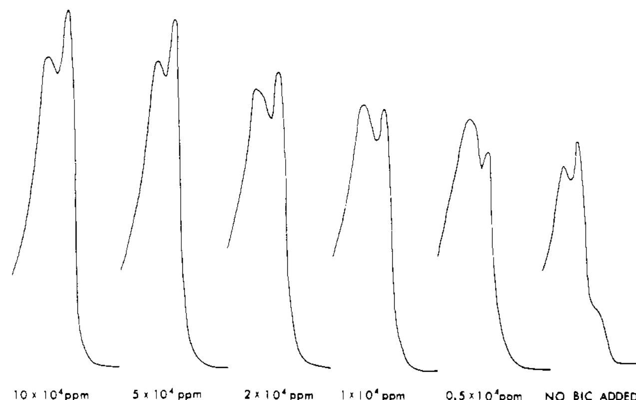

Figure 6. Absorption spectra of chloroform solutions containing MBC and different concentrations of BIC. To 9 ml of a 6.59-ppm MBC solution (equivalent to 10 ppm of benomyl), 1 ml of BIC chloroform solution was added. After 1 min the spectrum was recorded.

stays constant as long as the proportion of benomyl and MBC stays constant regardless of the actual concentrations of these two components.

Addition of *n*-Butyl Isocyanate (BIC) to MBC Solution to Produce Benomyl. Benomyl is produced quantitatively when sufficient BIC is added to a MBC solution. To determine the minimum concentration of BIC required, a standard MBC solution was prepared in

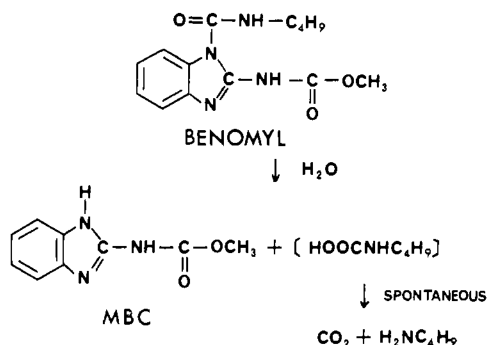

Figure 7. Degradation of benomyl in water.

chloroform (6.59 ppm). To nine portions of this solution was added one portion of a BIC–chloroform solution of known concentration.

One minute after mixing the solution was scanned. As shown in Figure 6, addition of 5×10^4 ppm of BIC solution, resulting in a BIC concentration of 0.5×10^4 ppm, was almost satisfactory. However, the completion of instantaneous reaction was ensured at a resulting BIC concentration of 1×10^4 ppm after adding 1 ml of 10×10^4 ppm of solution. The benomyl thus produced is stable in all solvents tested except methanol and ethanol.

DISCUSSION

When BIC is added to a MBC solution, it quantitatively re-forms benomyl and stabilizes it in organic solvents for an extended period. The graphic method of determining benomyl and MBC is less time consuming than the BIC method, but it is less accurate when the ratio of A/B is less than 0.80.

If the spectrophotometer available is of the nonautomatic scanning type, it is impossible to obtain continuous spectra of samples and the ratio of two maximum absorptions at 286 and 294 nm. However, the following describes the modified method suitable for routine analyses by using strictly 294 nm as the wavelength.

Standard curves for both benomyl and MBC must be prepared similarly as described in the aforementioned method. The absorptions of sample solution are first measured as usual (A) and then with BIC (A_{orig}) as described under Materials and Methods. The total concentration of benomyl (X_{orig}, ppm) will be obtained from the value of (A_{orig}) on the benomyl standard curve. The concentration of intact benomyl (X_{int}, ppm) in the solution then will be obtained from eq 2, which is derived from eq

$$X_{int} = \frac{X_{orig}(A - A_{MBC})}{A_{orig} - A_{MBC}} \qquad (2)$$

1, where A_{MBC} = absorption of MBC stoichiometrically equivalent to X_{orig} parts per million of benomyl, obtainable from the MBC standard curve.

Kilgore and White (1970) suggest that benomyl in aqueous media degrades according to the reaction shown in Figure 7. Chiba and Doornbos (1974) demonstrated that benomyl also decomposes to MBC in organic solvents, a result recently confirmed by Calmon and Sayag (1976a). One of the degradation products in organic solvents is *n*-butyl isocyanate (Figure 8) rather than *N*-butylcarbamic acid as occurs in water. The present study clearly demonstrates that BIC is stable in organic solvents and that the degradation reaction is reversible (Figure 8). In chloroform BIC was stable for at least 2 weeks. It is noteworthy that the concentration of BIC is the major factor in stabilizing benomyl in the organic solvents tested. Methanol and ethanol are exceptions; though rate of

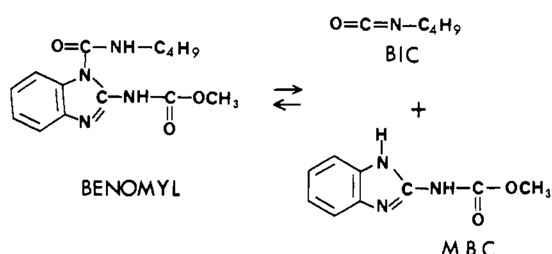

Figure 8. Degradation reaction of benomyl in common organic solvents.

degradation was reduced significantly, excess BIC did not stabilize benomyl in these solvents. Perhaps BIC forms corresponding urethanes with methanol and ethanol, thereby reducing the stabilizing action.

Calmon and Sayag (1976a,b) studied the kinetics and mechanism of the benomyl to MBC reaction, and reported that the conversion of benomyl to MBC proceeds by spontaneous intramolecular catalysis. The present experiments indicate that BIC is the key substrate controlling the rate of degradation of benomyl in organic solvents.

Once the benomyl–MBC reaction reached equilibrium in a given solvent, further degradation of benomyl was not observed. Hence, if all analyses are made after equilibrium has been reached, it will appear that benomyl is stable in that solvent as stated by Peterson and Edgington (1969). Many previous workers seemed to overlook the original degradation.

This method for determining benomyl is simple, rapid, and accurate. It has the further advantage of determining exact amounts of benomyl and MBC simultaneously in both aqueous and organic solutions. The method is not satisfactory for solutions containing large quantities of pigments or other coextractives from plants because these materials interfere with the spectrophotometric measurements necessary.

Because benomyl is not water soluble, a dispersing agent is required to form an aqueous suspension. Methanol (Clemons and Sisler, 1969), dimethyl sulfoxide (Hammerschlag and Sisler, 1972), surfactants (Reyes, 1975), wettable powders (Upham and Delp, 1973; Reyes, 1975; Rouchaud and Decallonne, 1974), and acetone (B. MacNeill, personal communication) have all been used. In the present study, methanol (0.1%) and Tween-20 (0.1 and 1%) were used. The results showed that the dispersing agents used affect the shape of the absorption curve and, accordingly, the quantitative determination of benomyl and MBC.

ACKNOWLEDGMENT

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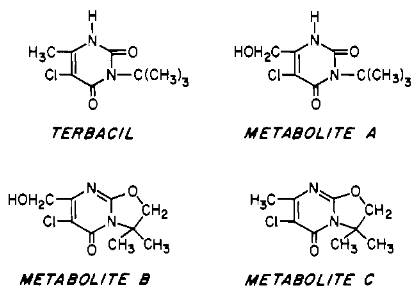
Determination of Terbacil and Metabolite Residues Using Microcoulometric Gas Chromatography

Richard F. Holt* and Harlan L. Pease

Residues of terbacil herbicide (3-*tert*-butyl-5-chloro-6-methyluracil) and three metabolites in plant and animal tissues and in soil are determined by initial extraction with chloroform, cleanup by liquid/liquid partitioning steps, and measurement by halogen-sensitive microcoulometric gas chromatography after formation of silyl derivatives of the metabolites. Method sensitivity is 0.04 ppm for all four compounds relative to a 25-g sample.

Terbacil (3-*tert*-butyl-5-chloro-6-methyluracil) is the active ingredient in Du Pont's Sinbar terbacil herbicide. An analytical method for determining residues of terbacil has been published previously (Pease, 1968). The present paper describes a new procedure which detects not only the parent compound but three metabolites: 3-*tert*-butyl-5-chloro-6-hydroxymethyluracil (metabolite A); 6-chloro-2,3-dihydro-7-hydroxymethyl-3,3-dimethyl-5*H*-oxazolo[3,2-*a*]pyrimidin-5-one (metabolite B); and 6-chloro-2,3-dihydro-3,3,7-trimethyl-5*H*-oxazolo[3,2-*a*]pyrimidin-5-one (metabolite C). These metabolites were all detected in the urine of dogs maintained on diets containing terbacil (Rhodes et al., 1969). The same metabolites were found in [^{14}C]terbacil studies with alfalfa (Rhodes, 1977).



This new residue method is based on gas chromatographic measurement after reaction of the extracted residues with Regisil-TMCS [bis(trimethylsilyl)trifluoroacetamide plus 1% trimethylchlorosilane]. The derivatization step is necessary to convert metabolites A and B to more volatile compounds.

EXPERIMENTAL SECTION

Apparatus and Reagents. The Tracor Model MT-220 gas chromatograph (Tracor Instruments, Austin, Tex.) equipped with a Dohrmann microcoulometric titrating system, consisting of a C-200 microcoulometer, T-300 halide titrating cell, and a S-200 sample inlet combustion unit was used for the analyses. The chromatographic column was 6 ft glass, 0.25 in. o.d., 3/16 in. i.d. packed with 5% XE-60/0.2% Epon 1001 on 80–100 mesh Gas-Chrom Q (Applied Science Lab., Inc., State College, Pa.).

Homogenization and extractions were conducted using a blender–centrifuge bottle and adapter base as shown in Figures 1 and 2. It is not necessary to construct this specialized equipment unless desired. However, use of this equipment reduces the possibility of mechanical losses by blending and centrifuging in the same piece of apparatus. Considerable analyst time is also saved as there is less handling of the sample and less cleanup of equipment. These items were designed in this laboratory and have been in use for several years. Conventional bottles and centrifuge tubes may be used but are somewhat more time consuming. Centrifugation was carried out with an International size 1, type SB centrifuge capable of accommodating the 250-ml bottle shown in Figure 1 and with a standard clinical model bench centrifuge.

The reference standards of terbacil and metabolites A, B, and C were obtained from the Biochemicals Department, Agrichemicals Marketing Division, E. I. du Pont de Nemours & Co., Inc., Wilmington, Del. The solvents used were distilled-in-glass purchased from Burdick and Jackson Laboratories, Inc., Muskegon, Mich. The silylation reagent used was Regisil-TMCS purchased from Regis Chemical Co., Chicago, Ill.

Isolation. Weigh 25 g of a representative sample (use 50 g when analyzing milk, urine, or aqueous solution) into the blender–centrifuge bottle, add 150 ml of chloroform, cover, place in the adapter base, and blend at high speed for about 5 min. (Note: Chloroform should be used only in a well-ventilated hood. Skin contact should be avoided. Use of neoprene gloves is suggested.) Centrifuge at 1500 rpm for 10 to 15 min and carefully pass the chloroform through cotton into a 500-ml round-bottomed flask. For liquid samples, the lower chloroform layer may be withdrawn from the blender bottle using a 200-ml syringe. Repeat the extraction two more times using additional 100-ml portions of chloroform. Add 10 ml of water to the combined extracts and evaporate the chloroform in a vacuum rotary evaporator at 60 °C.

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