mon ion (the direct transfer of $OH^+$, although unlikely (Jerina *et al.*, 1971), with generation of a cationoid intermediate serves as an acceptable and reasonable mechanism for this type of enzyme reaction) in the formation of the polar metabolites of aldrin. The aldrin ketone would be the resultant product of a NIH shift. The observed parallel enhancement in the production of dieldrin and *trans*-aldrin diol (TAD) by *p*-aminobenzoic acid, as indicated in Table III, is not inconsistent with the one enzyme proposal. The apparent stimulation by NADPH, which has been previously observed (Yu *et al.*, 1971), suggests a further resemblance to mixed function oxidase systems.

Aldrin alcohol formation is best explained by a simple hydration mechanism involving protonation and neutralization by a water molecule. This is not an uncommon reaction, even from a nonenzymatic viewpoint. Since the aldrin alcohol is not formed with the boiled enzyme blank, there appears to be an enzyme which can incorporate a proton ($H^+$) or equivalent species, also forming a cationoid-type intermediate. Subsequent neutralization of this intermediate would afford the aldrin alcohol (possibly both isomers (endo and exo) would be formed if the neutralization reaction is nonstereoselective). The apparent absence of an epoxide hydrase in these pea and bean root systems precludes the intermediacy of dieldrin in the formation of the diols and, therefore, simplifies the metabolic picture. It is likely that further studies of the metabolizing abilities of plants will reveal many metabolizing reactions exhibited by animal systems.

LITERATURE CITED

Brooks, G. T., in "Chlorinated Insecticides," CRC Press, Cleveland, Ohio, 1973, 300 pp.
Brooks, G. T., Harrison, A., *Biochem. Pharmacol.* 18(3), 557 (1969).
Jerina, D. M., Daly, J. W. Witkop, B., *Biochemistry* 10(3), 366 (1971).
Lichtenstein, E. P., Corbett, J. R., *J. Agr. Food Chem.* 17(3), 589 (1969).
Matthews, H. B., McKinney, J. D., The 5th International Congress on Pharmacology, San Francisco, Calif., July 1972.
McKinney, J. D., Fishbein, L., Barling, L., *Bull. Environ. Contam. Toxicol.* 7(1), 2 (1972).
McKinney, J. D., Keith, L. H., Alford, A., Fletcher, C. E., *Can. J. Chem.* 49(12), 1993 (1971).
McKinney, J. D., Oswald, E. O., Symposium on the NMR and Mass Spectra of Pesticides, Pesticides Division, 165th National Meeting of the American Chemical Society, Dallas, Texas, spring of 1973, Abstr. no. 56.
Mehendale, H. M., Skrentny, R. L., Dorough, H. W., *J. Agr. Food Chem.* 20(2), 398 (1972).
Mehendale, H. M., *Phytochemistry* 12(7), 1591 (1973).
Russell, D. W., Conn, E. E., Sutter, A., Grisebach, H., *Biochim. Biophys. Acta* 170, 210 (1968).
Yu, S. J., Kiigemagi, U., Terriere, L. C., *J. Agr. Food Chem.* 19(1), 5 (1971).

Received for review March 14, 1973. Accepted July 23, 1973.

# Characterization of Residues on Plants Following Foliar Spray Applications of Benomyl

Frederic J. Baude,* John A. Gardiner, and Jerry C. Y. Han

This paper reports the results of special tests using radiolabeled benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate] and methyl 2-benzimidazolecarbamate (MBC). These tests confirm by chemical means that the systemic fungicide benomyl has adequate stability in typical aqueous suspensions used for foliar applications. After application to plant foliage, benomyl constitutes a major component of the total residue for extended periods. Other than MBC, which also has fungicidal properties, no residues of possible conversion products of benomyl, particularly those which can form under alkaline conditions, were found in these tests. Thus, intact benomyl is available on treated plant surfaces for systemic fungus disease control. This finding supplements work reported elsewhere which has shown that more biologically active compound enters and moves within herbaceous plants when benomyl, rather than MBC, is applied to leaf surfaces.

Numerous recent literature accounts point to the instability of the systemic fungicide benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate] under certain conditions. Benomyl in solution and in plants has been reported to degrade to a fungitoxic degradation product known as MBC, methyl 2-benzimidazolecarbamate (Clemons and Sisler, 1969, 1970; Fuchs *et al.*, 1970; Kilgore and White, 1970; Maxwell and Brody, 1971; Ogawa *et al.*, 1971; Peterson and Edgington, 1969a,b; Sims *et al.*, 1969). The conversion of benomyl under alkaline conditions to 3-butyl-*s*-triazino[1,2a]benzimidazole-2,4(1*H*,3*H*)dione (STB) and 2-(3-butylureido)benzimidazole (BUB) has also been described (Ogawa *et al.*, 1971; White *et al.*, 1973).

This paper presents chemical evidence which shows that under simulated commercial use conditions for crop protection benomyl possesses excellent stability, both in aqueous suspension for spraying operations and as a residue on treated plants. It is thus available as an intact material for systemic fungus disease control.

E. I. du Pont de Nemours & Co., Inc., Biochemicals Department, Experimental Station, Wilmington, Delaware 19898.

EXPERIMENTAL SECTION

**Chemicals.** Analytical standards of benomyl and MBC were provided by E. I. du Pont de Nemours & Co., Inc., Biochemicals Dept., Wilmington, Del. [2-$^{14}$C]Benomyl (2.86 $\mu$Ci mg$^{-1}$, 0.830 $\mu$Ci $\mu$mol$^{-1}$, H. L. Klopping, unpublished data) had a radiochemical purity of >95% as estimated by dissolving a sample in chloroform, quickly

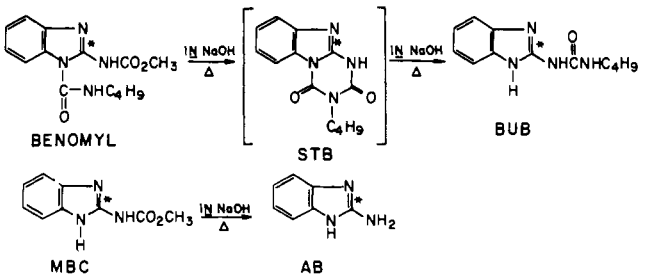


**Figure 1.** Analytical conversion reactions.

streaking aliquots and reference standards on a cellulose tlc plate (Merck, F-254, 100 $\mu$), and immediately developing to 10 cm with chloroform. Quantitation was performed by scraping intervals off the plate into vials, adding scintillation solution, and counting. Radiolabeled Benlate benomyl fungicide (0.397 and 0.920 $\mu$Ci mg$^{-1}$) was prepared by formulating portions of the [2-$^{14}$C]benomyl as a 52% wettable powder (52% active ingredient, 48% inert ingredients).

[2-$^{14}$C]MBC (4.74 $\mu$Ci mg$^{-1}$, 0.906 $\mu$Ci $\mu$mol$^{-1}$, H. L. Klopping, unpublished data) had a radiochemical purity of 98% as determined by dissolving [2-$^{14}$C]MBC in methanol, spotting an aliquot on a silica gel tlc plate (Analtech, F-254, 250 $\mu$), and developing to 10 cm with a solution of dioxane–formic acid (10:1 v/v). Quantitation was performed as described above for [2-$^{14}$C]benomyl.

2-Aminobenzimidazole (AB) was used directly as obtained commercially (Eastman Kodak).

BUB was prepared in the following manner. AB (13.3 g, 0.10 mol) and *n*-butylisocyanate (9.9 g, 0.10 mol) were stirred for 2 hr in toluene (200 ml). The mixture was refluxed for 3 hr, cooled, and the white precipitate filtered, washed with ether, and dried (22 g, 0.095 mol, 95%), mp 344–345° uncorr.

*Anal.* Calcd for $C_{12}H_{16}N_4O$ (232.3): C, 62.05; H, 6.95; N, 24.12. Found: C, 62.61; H, 6.87; N, 24.03.

STB was prepared in a two-step procedure. Benomyl (14.5 g, 0.050 mol) and *n*-butylisocyanate (19.8 g, 0.200 mol) were refluxed in toluene (200 ml) for 4 days. The toluene was stripped on a rotating evaporator and hexane (200 ml) was added to the oily residue. The white solid, 3-butyl-(1-butylcarbamoyl)-*s*-triazino[1,2a]benzimidazole-2,4(1*H*,3*H*)dione, was filtered off, washed with hexane, and dried (13.8 g, 0.035 mol, 70%), mp 96.5–97° dec.

*Anal.* Calcd for $C_{18}H_{23}N_5O_3$ (357.4): C, 60.49; H, 6.49; N, 19.53. Found: C, 60.90, 60.94; H, 6.32, 6.28; N, 19.56, 19.64.

This compound (3.0 g, 8.4 mmol) was refluxed in hexane (250 ml) for 2.5 hr. The white solid, STB, was filtered off, washed with hexane, and dried (1.8 g, 7.0 mmol, 83%), mp 289–290°.

*Anal.* Calcd for $C_{13}H_{14}N_4O_2$ (258.3): C, 60.46; H, 5.46; N, 21.69. Found: C, 60.86, 60.58; H, 5.18, 4.77; N, 21.47, 21.29.

The chemical identity of both BUB and STB was confirmed by mass spectroscopic analysis.

The fungicides basic copper sulfate (Phelps Dodge Basic Copper Sulfate, Phelps Dodge) and lime sulfur (Security Lime Sulfur soln., 30%, Woolfolk Chemical Works, Ltd.) were used as obtained commercially, but were diluted as recommended by the manufacturer.

**Equipment.** Samples were counted for $^{14}$C using a Nuclear-Chicago liquid scintillation system model 6801. A Varian Aerograph/Berthold Model 6000-2 automatic/integrating tlc radioscanner was used to detect and measure $^{14}$C on tlc plates. Samples were combusted for total $^{14}$C in a Packard Tri-Carb model 305 sample oxidizer.

**General Analytical Approach.** Residue methods normally involve the dissolution of residues into a solvent as an initial step. However, the differentiation and quantitation of benomyl from MBC on sprayed plant surfaces by such techniques are precluded, since MBC will continuously form from residual benomyl while in solution at these levels. In most situations, the work-up steps take so long and the dilutions are so great that all the residual benomyl may be converted to MBC.

Therefore, a research method for this determination, using [$^{14}$C]benomyl, was developed which involves first macerating the crop tissue in 1 *N* aqueous sodium hydroxide and immediately refluxing the mixture. Any [$^{14}$C]benomyl present will be converted to a stable derivative [$^{14}$C]BUB, and concomitantly, any [$^{14}$C]MBC present will be converted to a different stable derivative [$^{14}$C]AB. Figure 1 shows the conversion reactions in this method. STB, if present, is not stable in the hot base and is converted to [$^{14}$C]BUB along with benomyl.

A second and separate method of analysis is used to rule out the initial presence of not only STB but also BUB and AB. The final amounts of BUB and AB after NaOH reflux are then reflective of the original residues of benomyl and MBC, respectively.

After the NaOH reflux procedure is used to make the stable derivatives of benomyl and MBC, the extracts are then handled by regular partition cleanup and concentration techniques. The [$^{14}$C]BUB and [$^{14}$C]AB are separated by tlc and quantitated by radioscanning or radioautography and liquid scintillation counting.

**Table I. Method Evaluation Studies, NaOH Reflux Method**

| Substrate | % $^{14}$C added | | % $^{14}$C found | |
|---|---|---|---|---|
| | Benomyl | MBC | BUB | AB |
| None | 100 | 0 | 94 | 6 |
| None | 100 | 0 | 97 | 3 |
| None | 100 | 0 | 96 | 4 |
| None | 0 | 100 | 0 | 100 |
| None | 0 | 100 | 0 | 100 |
| Cucumber leaves | 100 | 0 | 91 | 9 |
| Cucumber leaves | 68 | 32 | 74 | 26 |
| Cucumber leaves | 38 | 62 | 53 | 47 |
| Cucumber leaves | 14 | 86 | 23 | 77 |
| Cucumber leaves | 0 | 100 | 5 | 95 |
| Apple leaves | 64 | 36 | 67 | 33 |
| Apple leaves | 0 | 100 | 0 | 100 |

**Method Evaluation Studies.** Table I gives the results of studies to determine the validity of the NaOH reflux method for distinguishing between benomyl and MBC. For recovery purposes, [2-$^{14}$C]benomyl was normally added to the systems from a freshly made, concentrated chloroform solution. [2-$^{14}$C]MBC was added by methanol solution. In both cases the solvents were evaporated quickly prior to proceeding.

In all cases, samples were refluxed for about 1 hr in about 1 *N* NaOH before anything else was done. Also, in all cases, the calculated concentration of benomyl in the NaOH refluxing solution was no more than about 100 $\mu$g/ml, otherwise conversion of benomyl to BUB becomes less and less complete; alternate degradations can take place, *e.g.*, degradation of benomyl to MBC and AB instead of BUB.

After the reflux step the method was operated in different ways, depending on the nature of the sample. It was sometimes possible to analyze aliquots of the NaOH solutions directly by tlc using $^{14}$C-readout techniques. When green plant tissue samples were involved, the BUB and AB in the refluxed solutions were extracted into three or four portions of ethyl acetate, which was concentrated and analyzed. Hexane washes of the basic solutions, prior to ethyl acetate extraction, were used occasionally as an additional clean-up step.

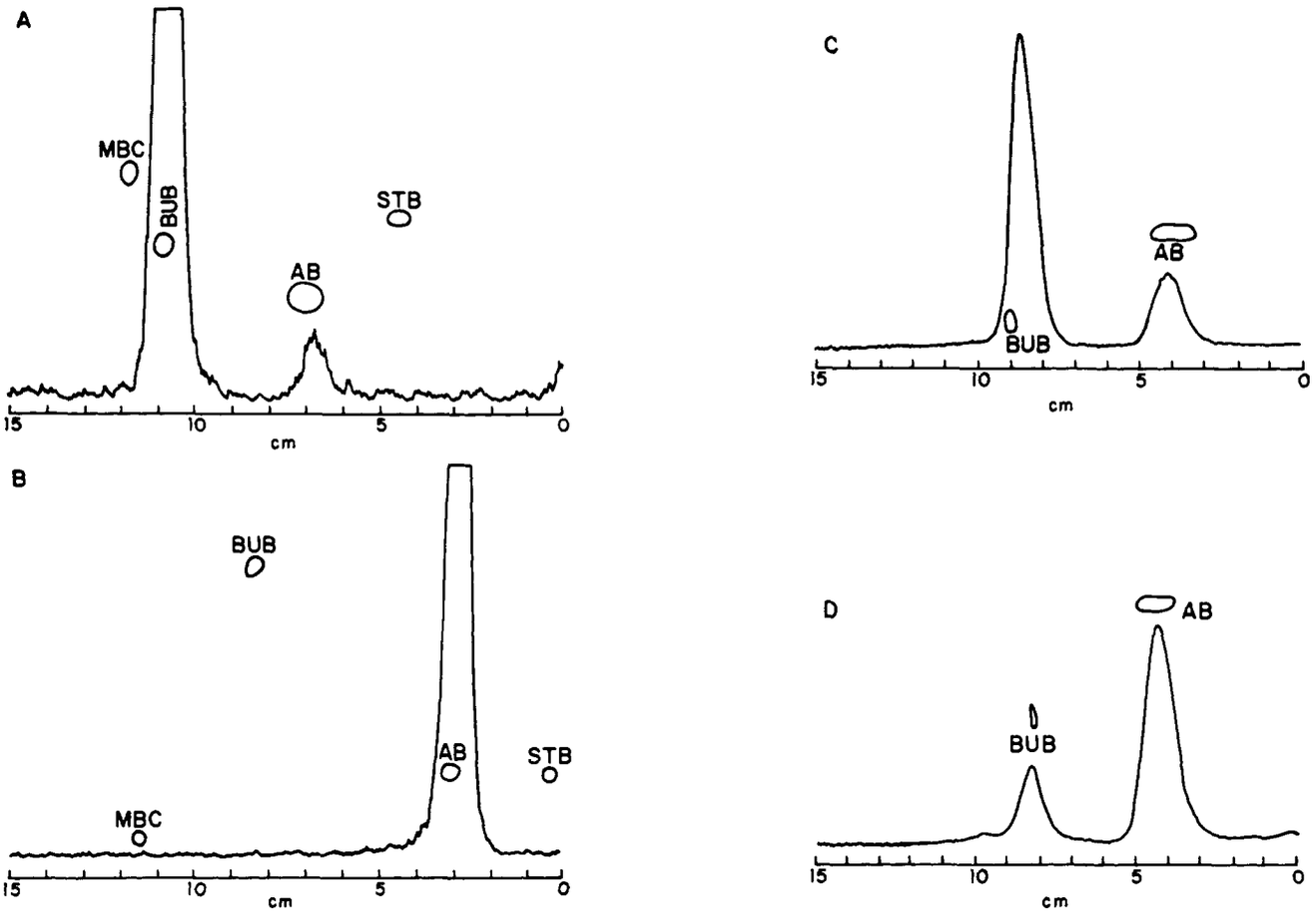


**Figure 2.** Radioscans from NaOH reflux method evaluation studies. Scan A, benomyl only, no substrate; Scan B, MBC only, no substrate; Scan C, 68% benomyl, 32% MBC added to cucumber leaves; Scan D, 14% benomyl, 86% MBC added to cucumber leaves.

As shown in Table I, the conversion of pure [2-$^{14}$C]benomyl to [$^{14}$C]BUB, even under the best conditions, is not strictly quantitative. Usually, the conversion is at least 90% (see Figure 2). The remainder of the $^{14}$C is found as AB. On the other hand, pure [2-$^{14}$C]MBC converts to AB quantitatively (Figure 2). Only in one case was any [$^{14}$C]BUB (5%) found from [$^{14}$C]MBC by tlc analysis; this was apparently an artifact in the one particular run.

The data in Table I for mixtures of benomyl and MBC show the experimental discrepancies between added amounts of benomyl and the detected amounts, determined as BUB (Figure 2 has typical scans). Evidently these differences occur because the conversion to BUB is not absolutely quantitative and because, for crop analysis, the ethyl acetate partition steps had to be used for cleanup and concentration of residue. In these partitioning steps, the BUB and AB products must pass into the organic phase to exactly the same degree to get the best answer. Apparently, this was not always the case.

*In spite of the difficulties with precise quantitation*, the NaOH reflux method is clearly capable of qualitatively distinguishing between benomyl and MBC. While it should be viewed as a semiquantitative method at best, at the present time it is the most reliable way of distinguishing between benomyl and MBC at residue levels on natural substrates.

**[$^{14}$C]Benlate Benomyl Fungicide in Aqueous Suspension.** [$^{14}$C]Benlate (0.920 $\mu$Ci mg$^{-1}$) was suspended in tap water (25 ml, 1200 ppm of product, 600 ppm active, 1 lb of product/100 gal) in a covered beaker, initial pH 7.3. The suspension was stirred continually at room temperature (23°) and after 0, 1, 6, 25, and 49 hr, aliquots were streaked directly on two tlc plates (Silica Gel, Merck F-254) containing a fluorescent indicator. The reference compounds (AB, BUB, MBC, and STB) in methanol were spotted at the origins. One plate was developed to 15 cm with a solution of ethyl acetate, methanol, and ammonium hydroxide (100:25:1 v/v/v), which separates STB and AB from MBC and BUB. The other plate was developed to 15 cm with a solution of ethyl acetate, dioxane, methanol, and ammonium hydroxide (32:4:1:1 v/v/v/v) to separate BUB from MBC. Benomyl, which has an $R_f$ slightly higher than MBC in both systems, continually decomposes to MBC during tlc.

At all but one of the same time intervals mentioned, a second aliquot (2 ml) was refluxed in 1 *N* aqueous sodium hydroxide (10 ml) for 1 hr. Aliquots of the cooled solution were streaked directly on a tlc plate (Silica Gel, Merck F-254) containing fluorescent indicator. The reference compounds (AB and BUB) in methanol were spotted on the origin. The plate was developed for 15 cm in an ethyl acetate–methanol–ammonium hydroxide solution (100:25:1 v/v/v) which separates BUB from AB.

The developed plates were quantitated using the Varian Radio Scanner equipped for integration to detect position and relative amounts of $^{14}$C activity in comparison to the reference compounds.

Table II shows the results for all time intervals. Figure 3 shows the radioscans from the 6-hr interval for illustration.

**Greenhouse Exposure Tests of [$^{14}$C]Benlate Benomyl Fungicide on Beans in the Presence of Alkaline Pesticides.** Eight pots, each with nine actively growing pinto beans 8 in. high with the third trifoliate leaves fully expanded, were maintained in a greenhouse and were watered at the soil surface. The temperature ranged from 70 to 95°F during the test period.

Plants in four of the pots were sprayed with basic cop-

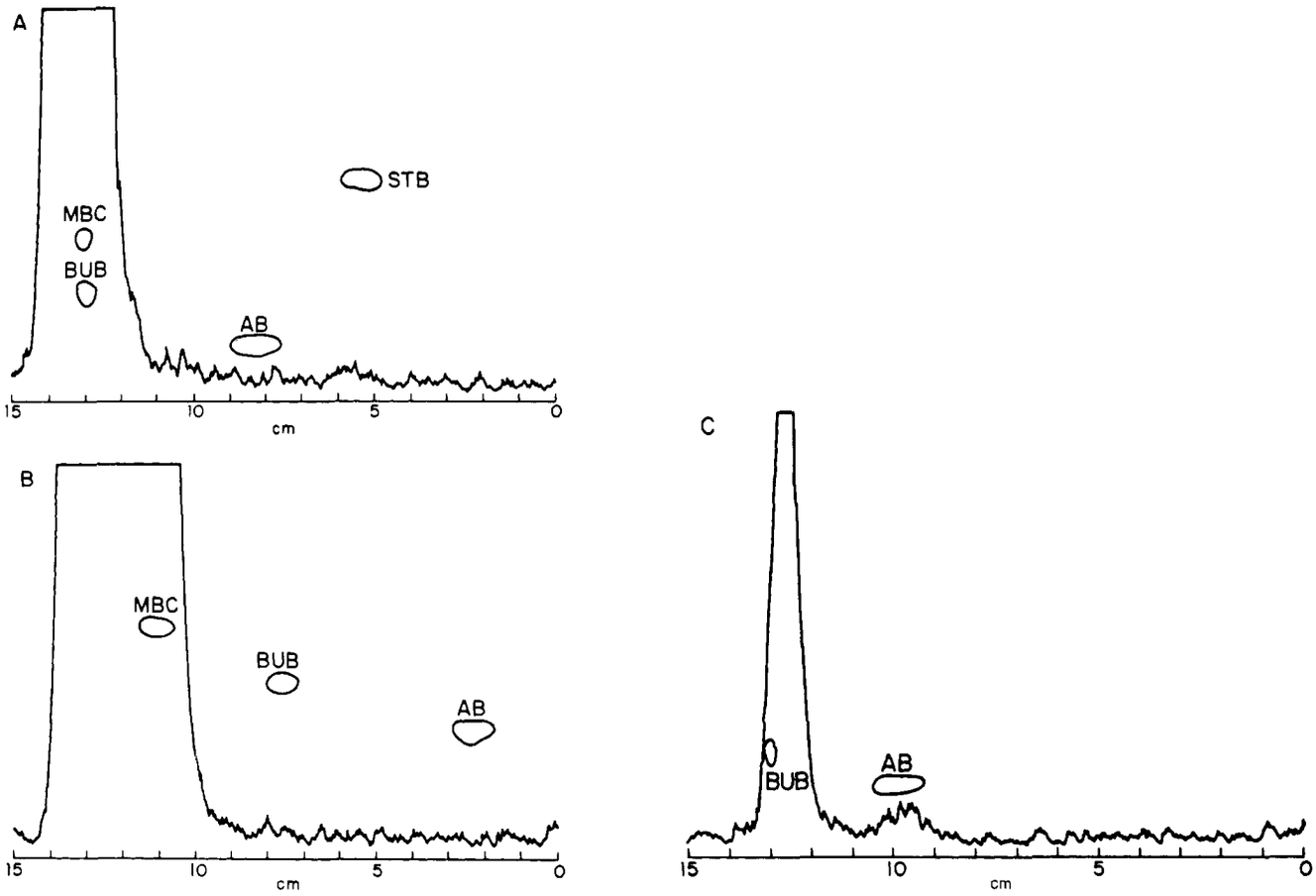


**Figure 3.** Radioscans from aqueous suspension studies. Scan A, 6-hr exposure; direct analysis with tlc plate developed in EtOAc–MeOH–$NH_4OH$ (100:25:1); Scan B, 6-hr exposure; direct analysis with tlc plate developed in EtOAc–dioxane–MeOH–$NH_4OH$ (32:4:1:1); Scan C, 6-hr exposure, NaOH reflux method.

**Table II. Benlate in Aqueous Suspension (23°) (1.0 lb of Product/100 gal)**

| | | By NaOH reflux method | | By direct tlc method | | | |
|---|---|---|---|---|---|---|---|
| Hours | pH | % benomyl[a] | % MBC | % benomyl–MBC | % AB | % BUB | % STB |
| 0 | 7.3 | 97 | 3 | >99 | <0.1 | <0.1 | <0.1 |
| 1 | 7.3 | 90 | 10 | >99 | <0.1 | <0.1 | <0.1 |
| 6 | 7.8 | 93 | 7 | >99 | <0.1 | <0.1 | <0.1 |
| 25 | 7.6 | | | >99 | <0.1 | <0.1 | 0.2 |
| 49 | 8.0 | 95 | 5 | 99 | <0.1 | <0.1 | 0.9 |

[a] Not corrected for incomplete conversion to BUB; the NaOH method was used only to tell the relative amounts of benomyl *vs.* MBC.

per sulfate in water (8 lb of product/100 gal, pH 8.0) and the plants in the other four pots were sprayed with lime sulfur in water (1% active solution, pH 10.1). The applications were made by a small, $CO_2$-pressurized hand sprayer. One week later, [$^{14}$C]Benlate (0.920 $\mu$Ci mg$^{-1}$), suspended in tap water (2 lb of product/100 gal), was sprayed on all the plants. A second application of each alkaline pesticide was made on the respective bean plants 1 week after the [$^{14}$C]Benlate spray.

Foliage samples for analysis were taken 3 days after the [$^{14}$C]Benlate application, and at 1 day, 1 week, and 3 weeks after the second alkaline pesticide application.

About 30 g (fresh weight) of each sample was macerated in a blender with methanol (100 ml). The extracts were filtered and the residue washed in additional methanol (50 ml). The remaining plant substrate was air-dried and analyzed for unextracted $^{14}$C by total combustion. The extracts were combined, and extracted $^{14}$C activity was determined by scintillation counting. Extracts were then stripped to dryness under vacuum on a rotating evaporator at 40°. The residues were taken up in methanol (5 ml) with no loss of $^{14}$C. Aliquots were streaked on tlc plates as already described. The reference compounds (AB, BUB, MBC, and STB) in methanol were spotted on the origin. The tlc plates were developed for 15 cm in an ethyl acetate–dioxane–methanol–ammonium hydroxide solution (32:4:1:1 v/v/v/v), which served in this case to adequately separate all the reference compounds. Quantitation proceeded by radioscanning.

Another portion (about 30 g) of each foliage sample was refluxed without prior treatment in 1 *N* aqueous sodium hydroxide (100 ml) for 1 hr. The mixture was cooled, extracted with ethyl acetate (3 × 150 ml), and the combined extracts were stripped on a rotating evaporator. The residue was taken up in methanol (5 ml) and aliquots were analyzed by tlc as before. However, in this case, plates were developed in ethyl acetate, methanol, and glacial acetic acid (320:80:1 v/v/v).

Results appear in Tables III and IV. Figure 4 shows representative radioscans from the lime sulfur test series. About 2–6% of the total $^{14}$C from the methanol extracts remained near the origins of the tlc plates. These origin

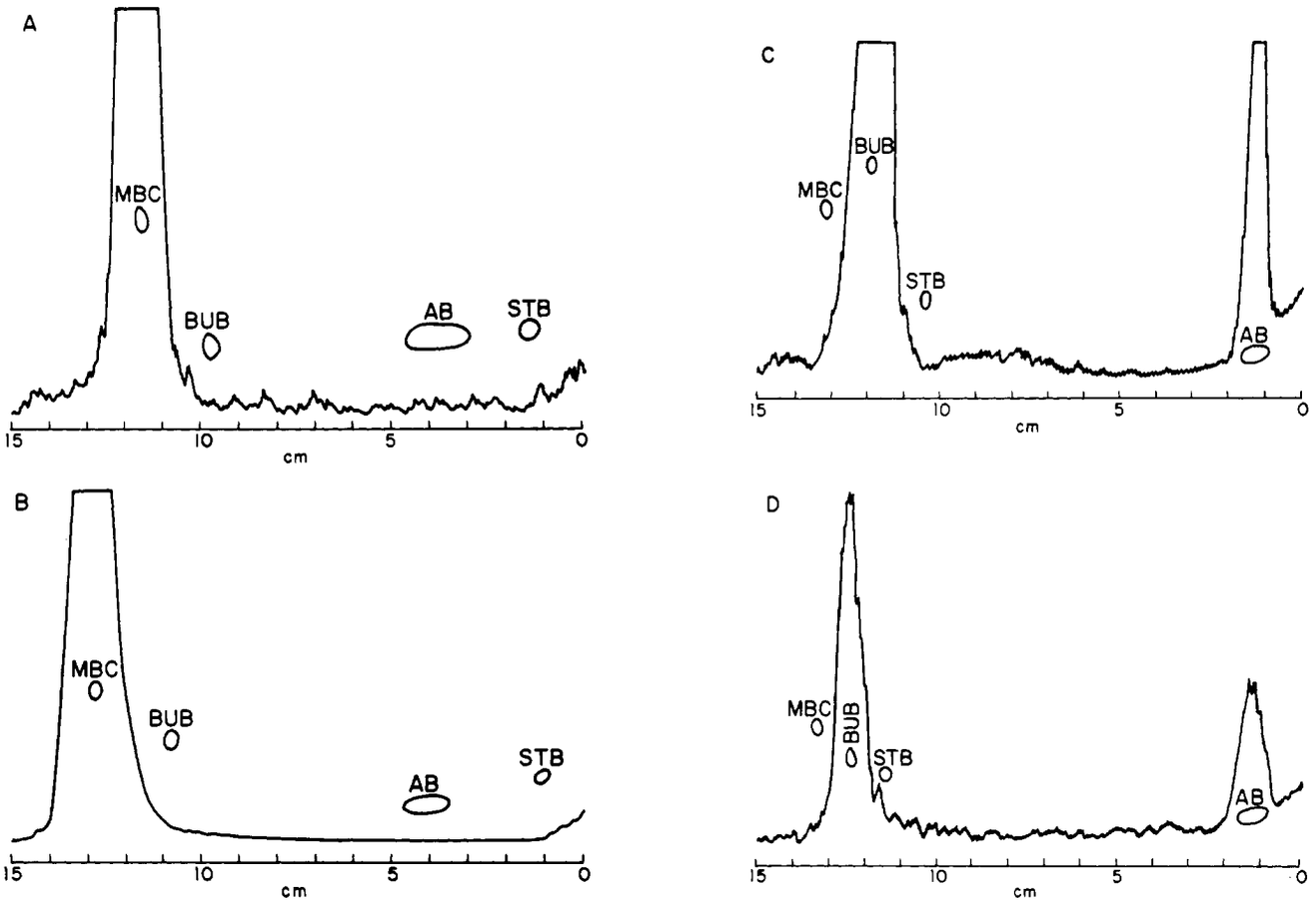


**Figure 4.** Radioscans from greenhouse [$^{14}$C]Benlate lime sulfur alternate spray tests: Scan A, 3 days after Benlate spray, MeOH–tlc method; Scan B, 28 days after Benlate spray (21 days after second lime sulfur treatment), MeOH–tlc method; Scan C, 3 days after Benlate spray, NaOH reflux method; Scan D, 28 days after Benlate spray (21 days after second lime sulfur treatment, NaOH reflux method.

**Table III. [$^{14}$C]Benlate and Basic Copper Sulfate Alternate Spray Test, Greenhouse Exposure**

| Sampling time relative to Benlate spray | Percentage of total $^{14}$C residue extracted by MeOH method | By NaOH reflux method[a] | | By direct MeOH–tlc method | | | |
|---|---|---|---|---|---|---|---|
| | | % benomyl | % MBC | % benomyl–MBC | % AB | % BUB | % STB |
| 3 days after | 98 | | | >95 | <1 | <1 | <1 |
| 8 days after[b] | 98 | 60 | 40 | >95 | <1 | <1 | <1 |
| 14 days after | 96 | 65 | 35 | >95 | <1 | <1 | <1 |
| 28 days after | 96 | 67 | 33 | >95 | <1 | <1 | <1 |

[a] The NaOH method was used only to tell the relative amounts of Benomyl *vs.* MBC. [b] The second basic copper sulfate spray was applied 7 days after the Benlate spray.

**Table IV. [$^{14}$C]Benlate and Lime Sulfur Alternate Spray Test, Greenhouse Exposure**

| Sampling time relative to Benlate spray | Percentage of total $^{14}$C residue extracted by MeOH method | By NaOH reflux method[a] | | By direct MeOH–tlc method | | | |
|---|---|---|---|---|---|---|---|
| | | % benomyl | % MBC | % benomyl–MBC | % AB | % BUB | % STB |
| 3 days after | 96 | 75 | 25 | >95 | <1 | <1 | <1 |
| 8 days after[b] | 97 | 70 | 30 | >95 | <1 | <1 | <1 |
| 14 days after | 95 | 60 | 40 | >95 | <1 | <1 | <1 |
| 28 days after | 97 | 65 | 35 | >95 | <1 | <1 | <1 |

[a] The NaOH method was used only to tell the relative amounts of benomyl *vs.* MBC. [b] The second lime sulfur spray was applied 7 days after the Benlate spray.

areas were removed from the plates and treated with hot methanol for 20 min to dissolve the $^{14}$C residue, which was then rechromatographed. At least 50% of the originally retained $^{14}$C was benomyl and/or MBC. Again, no STB or BUB was detected.

**Outdoor Exposure Tests of [$^{14}$C]Benlate Benomyl**

**Table V. [$^{14}$C]Benlate on Various Plant Seedlings, Outdoor Exposure**

| Time after spraying, days | Total rainfall, in. | Total $^{14}$C detected, $\mu$Ci | By NaOH reflux method[a] % benomyl | % MBC |
|---|---|---|---|---|
| Apple leaves | | | | |
| 0 | Trace | 0.87 | 91 | 9 |
| 3 | 0.2 | 0.35 | 91 | 9 |
| 7 | 5.0 | 0.45 | 91 | 9 |
| 13 | 5.6 | 0.34 | 81 | 19 |
| 21 | 5.6 | | 48 | 52 |
| Cucumber leaves | | | | |
| 0 | 0 | 0.65 | 91 | 9 |
| 3 | 0 | 0.59 | 60 | 40 |
| 9 | Trace | 0.40 | 55 | 45 |
| 17 | Trace | 0.14 | 71 | 29 |
| 23 | 0.4 | 0.06 | 60 | 40 |
| Banana leaf | | | | |
| 0 | 0 | 1.04 | 91 | 9 |
| 3 | 0 | 0.60 | 86 | 14 |
| 9 | Trace | 0.62 | 86 | 14 |
| 17 | Trace | 0.28 | 75 | 25 |
| 23 | 0.4 | 0.36 | 77 | 23 |
| Orange leaves | | | | |
| 23 | 0.4 | 0.12 | 53 | 47 |
| Grape leaves | | | | |
| 21 | 5.4 | | 62 | 38 |

[a] The NaOH method was used only to tell the relative amounts of benomyl *vs.* MBC.

**Fungicide on Plants.** Five potted apple seedlings (2 to 3 ft high), 32 2-week-old cucumber seedlings, a banana seedling (4 ft high), three orange seedlings (2 to 3 ft high), and three grape plants (about 2 ft high) were sprayed to runoff with [$^{14}$C]Benlate (0.397 $\mu$Ci mg$^{-1}$) suspended in water (12 oz/100 gal). The plants were exposed outdoors in Delaware in an open sunny location for up to 23 days.

For analyses, five apple leaves (*ca.* 1.8 g), eight cucumber leaves (*ca.* 3.0 g), one piece of a banana leaf of intermediate age halved along its axis (*ca.* 12 g), six orange leaves (*ca.* 1.6 g), and six grape leaves were taken at various time intervals. The fresh plant leaf samples were macerated in 1 *N* aqueous sodium hydroxide (100 ml) using a blender. The macerates were refluxed with stirring for 1 hr. The cooled mixtures were centrifuged to remove the solids. In some tests (cucumber, banana, and orange) the solids were air-dried and combusted for unextracted $^{14}$C. Extraction efficiency was between 83 and 98% and averaged 92%. The decantates were counted for total $^{14}$C, and then extracted with ethyl acetate (3 × 150 ml). The combined ethyl acetate extracts were dried over anhydrous sodium sulfate and filtered. The filtrate was stripped under vacuum on a rotating evaporator and the residue taken up in methanol (1 ml). Aliquots were streaked onto tlc plates as already described. The reference compounds (AB and BUB) in methanol were spotted on the origins.

The tlc plates were developed for 15 cm in either an ethyl acetate–methanol–ammonium hydroxide solution (100:25:0.5 v/v/v) or in ethyl acetate–dioxane–methanol–ammonium hydroxide solution (320:40:10:1 v/v/v/v). Either developing solution serves to adequately separate AB from BUB. The areas of $^{14}$C activity were located with radioautograms (Kodak No-Screen X-ray film) and quantitation followed by scraping the $^{14}$C areas corresponding to the reference compounds and subsequent liquid scintillation counting. Some plates were also scanned by the Varian tlc radioscanner.

The results appear in Table V along with the rainfall record. A typical radioscan is shown in Figure 5.

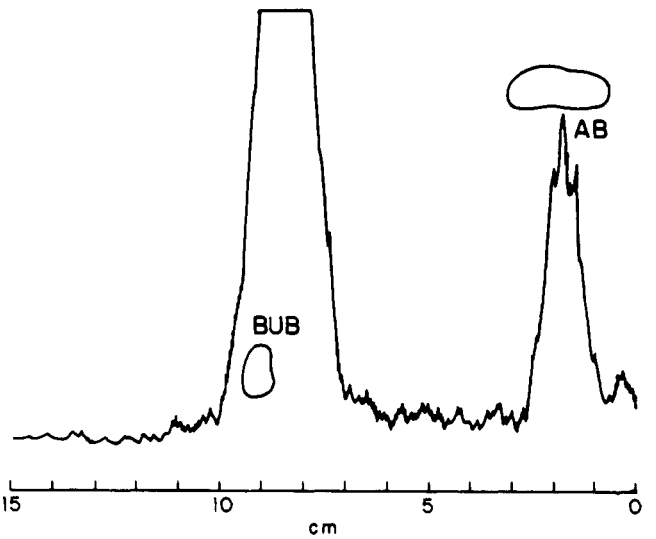


**Figure 5.** Radioscan from outdoor exposure test. Scan of banana leaf extract, 23 days exposure, NaOH reflux method.

**Field Exposure Test of [$^{14}$C]Benlate Benomyl Fungicide on Bean Plants.** A plot of bean plants (*Phaseolus vulgaris* L. "Tendergreen") was grown at a test farm in Delaware. At about 75% bloom, a 10-ft row was sprayed with a suspension of [$^{14}$C]Benlate (0.920 $\mu$Ci mg$^{-1}$) in tap water (1 lb of product/acre). One week later, somewhat past 100% bloom, the treatment was repeated. Leaf samples were taken for analysis at selected intervals and immediately frozen. Separate bean fruit samples were also collected and analyzed.

Leaf and fruit subsamples, about 13–20 g, were macerated in 1 *N* aqueous sodium hydroxide (100 ml) and the mixture refluxed for 1 hr. The previously described analytical procedures were then applied to determine % benomyl *vs.* % MBC. The NaOH solutions were also counted for $^{14}$C to determine total $^{14}$C residue. Results are shown in Table VI.

A separate leaf subsample (12.9 g), taken 1 week after the first spray, was stirred in methanol (100 ml) for 24 hr. The green supernatant was stripped on a rotating evaporator and the residue extracted with methanol (5 ml). Aliquots were analyzed by tlc. The areas of $^{14}$C activity were visualized with X-ray film and subsequently quantitated by scraping off the areas of interest and scintillation counting. Benomyl–MBC constituted 80% and AB, BUB, and STB were each less than 2% of the total $^{14}$C detected on the tlc plate. The solids remaining after the MeOH surface rinse treatment were air-dried and combusted for unextracted $^{14}$C. The rinse procedure removed 53% of the total $^{14}$C.

Similarly, a separate fruit subsample (39.7 g), taken 1 week after the second spray, was stirred in refluxing methanol (150 ml) for 1 hr. The green supernatant was stripped on a rotating evaporator and the residue extracted with methanol (5 ml). Aliquots were analyzed by tlc. Quantitation proceeded as before. Benomyl–MBC constituted 80% and AB, BUB, and STB were each less than 2% of the total $^{14}$C detected on the tlc plate. The dried plant residue after solvent extraction was combusted for unextracted $^{14}$C. The reflux procedure removed 56% of the total $^{14}$C.

## RESULTS AND DISCUSSION

Studies found elsewhere (Upham and Delp, 1973) compare the relative merits of benomyl *vs.* MBC for foliar applications from a biological standpoint. Use of either compound in foliar spray programs involves particulate materials because of the extremely low water solubilities. To understand the differences in biological performance, it is pertinent to study the chemical stability of benomyl

**Table VI. [$^{14}$C]Benlate on Bean Plants, Field Exposure**

| Sampling time | Total rainfall[a] in. | ppm total $^{14}$C residue[b] | By NaOH reflux method[c] % benomyl | % MBC |
|---|---|---|---|---|
| Leaves | | | | |
| 1/2 hr after first spray | 0.0 | 53 | 98 | 2 |
| 7 days after first spray | 2.0 | 4.5 | 78 | 22 |
| 1/2 hr after second spray[d] | 2.0 | 55 | 97 | 3 |
| 7 days after second spray | 2.5 | 20 | 78 | 22 |
| Bean fruit | | | | |
| 7 days after first spray | 2.0 | 0.3 | 38 | 62 |
| 7 days after second spray[d] | 2.5 | 1.7 | 42 | 58 |

[a] Rainfall plus overhead sprinkler system. [b] Expressed as benomyl. [c] The NaOH method was used only to tell the relative amounts of benomyl *vs.* MBC. [d] Second spray applied 7 days after first spray.

under these use conditions. The chemical results are presented separately to clarify the stability of benomyl relative to conversion to MBC when used for foliar applications according to product label directions.

By use of the NaOH reflux technique and radiolabeled materials, benomyl residues can be distinguished from MBC residues (Table I). The results in Table II show that greater than 90% of the original [2-$^{14}$C]benomyl was still intact in a Benlate preparation held in aqueous suspension for 49 hr at 23°. The results in Table II are not corrected for the less than quantitative conversion of benomyl to the BUB derivative in the reflux method employed. Tests of this type confirm that intact benomyl possesses sufficient stability in aqueous suspension to allow normal spraying operations to be made.

The results in Tables III–VI from both greenhouse and outdoor plant residue studies with [2-$^{14}$C]benomyl formulated as Benlate are semiquantitative in nature, since they are also based on the NaOH reflux method. They provide, however, firm evidence that benomyl remains as a major entity for extended periods of time on foliar-treated plants, even when exposed to sunlight and rainfall in the field.

Benomyl was estimated at 48, 60, 77, 53, and 62%, respectively, of the composite benomyl plus MBC residue on the leaves of apple, cucumber, banana, orange, and grape plants 21–23 days after treatment under outdoor conditions (Table V). During this study the total $^{14}$C residues, including unextractable $^{14}$C, on the treated cucumber and banana leaves decreased to about 10–35% of the original amount applied, as shown in the table. The results on bean plants under actual field conditions appear quite similar (Table VI).

In Tables III and IV, the data from greenhouse residue studies with [2-$^{14}$C]benomyl formulated as Benlate show that when benomyl is subjected to the pre- or postinfluence of two selected alkaline pesticides, basic copper sulfate and lime sulfur, no spurious leaf surface conversion products of benomyl, in particular STB or BUB, were detected. Likewise, no STB or BUB (<2%) was observed in additional samples from the field test.

Taken together, these laboratory, greenhouse, and field evaluations of the chemical nature of the residues on plant foliage following Benlate benomyl fungicide treatments indicate that intact benomyl itself is a major residual component. These data thus provide direct evidence of a basic chemical difference between benomyl foliar treatments to plants *vs.* treatments with MBC alone.

Work by Siegel and Zabbia (1972) plus several earlier reports indicate that material which moves within plants by systemic means may be mostly MBC plus smaller amounts of AB. However, the overall effectiveness of Benlate benomyl fungicide, particularly in recommended foliar applications, appears to depend on benomyl as well as the MBC degradation product. The role that benomyl plays in the control of fungi and mites on herbaceous plants (*vs.* that of MBC) was studied by Upham and Delp (1973). These workers found that direct foliar applications of [2-$^{14}$C]MBC resulted in much less (−20×) plant penetration of biologically active component than similar treatments with [2-$^{14}$C]benomyl. Fuchs *et al.* (1972) have observed differences in release and movement of active component in benomyl root-treated *vs.* MBC root-treated plants. Hammerschlag and Sisler (1972, 1973) have reported on differences in the response of *S. pastorianus*, *S. cerevesiae*, and *U. maydis* to benomyl *vs.* MBC. Finally, Solel and Edgington (1973) have recently reported on the transcuticular movement of benomyl *vs.* MBC.

Several years ago, Clemons and Sisler (1969) speculated that the improved performance of benomyl over MBC might be due to superior penetration properties. Both biological and chemical data appear to support this explanation.

ACKNOWLEDGMENT

The authors gratefully acknowledge B. I. Dittmar for preparing the sample of BUB and B. L. Richards, Jr., for maintaining many of the seedlings.

LITERATURE CITED

Clemons, G. P., Sisler, H. D., *Phytopathology* **59,** 705 (1969).
Clemons, G. P., Sisler, H. D., *Pestic. Biochem. Physiol.* **1,** 32 (1970).
Fuchs, A., Homans, A. L., deVries, F. W., *Phytopathol. Z* **69,** 330 (1970).
Fuchs, A., Vandenberg, G. A., Davidse, L. C., *Pestic. Biochem. Physiol.* **2,** 191 (1972).
Hammerschlag, R. S., Sisler, H. D., *Pestic. Biochem. Physiol.* **2,** 123 (1972).
Hammerschlag, R. S., Sisler, H. D., *Pestic. Biochem. Physiol.* **3,** 42 (1973).
Kilgore, W. W., White, E. R., *Bull. Environ. Contam. Toxicol.* **5,** 67 (1970).
Maxwell, W. A., Brody, G., *Appl. Microbiol.* **21,** 944 (1971).
Ogawa, J. A., Bose, E., Manji, B. T., White, E. R., Kilgore, W. W., *Phytopathology* **70,** 905 (1971).
Peterson, C. A., Edgington, L. V., *Phytopathology* **59,** 1044 (1969a).
Peterson, C. A., Edgington, L. V., *J. Agr. Food Chem.* **17,** 898 (1969b).
Siegel, M. R., Zabbia, A. J., Jr., *Phytopathology* **62,** 630 (1972).
Sims, J. J., Mee, H., Erwin, D. C., *Phytopathology* **59,** 1775 (1969).
Solel, Z., Edgington, L. V., *Phytopathology* **63,** 505 (1973).
Upham, P. M., Delp, C. J., *Phytopathology* **63,** 814 (1973).
White, E. R., Bose, E. A., Ogawa, J. M., Manji, B. T., Kilgore, W. W., *J. Agr. Food Chem.* **21,** 616 (1973).

Received for review June 11, 1973. Accepted August 10, 1973.