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Assessing *N,N′*-Dibutylurea (DBU) Formation in Soils after Application of *n*-Butylisocyanate and Benlate Fungicides

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N,N′-Dibutylurea (DBU) is a breakdown product of benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazole carbamate], the active ingredient in Benlate fungicides, and has been proposed as one cause for crop damage that growers claim to have occurred from the use of Benlate 50 DF fungicide. This study assessed DBU formation upon (1) application of *n*-butyl-1-[^{14}C]butylisocyanate (BIC), the immediate precursor to DBU formation, in four soils at two water potentials (0.03 and 0.1 MPa) and (2) application of benomyl *butyl*-1-^{14}C-benomyl enriched Benlate DF and SP fungicides to two soils at various combinations of negative water potential (0.03 or 0.1 MPa) and temperature (23 or 33 °C). Parent compounds, metabolites, and $^{14}CO_2$ were tracked using chromatographic analysis with radioassay and UV detection, liquid scintillation counting, and postextraction oxidation of the soil. At 0.03 MPa in all four BIC-treated soils, DBU formation was never detected. At 0.1 MPa, DBU was detected in two soils, but at concentrations <3.6 μg kg^{-1} (0.3 wt % of applied BIC). In both soils treated with benomyl formulations, DBU formation was observed with only Benlate 50 DF application at 0.03 MPa and 23 °C, which was followed by rapid dissipation of DBU. The maximum concentration observed was 0.41 μg g^{-1} (0.65 wt % of applied benomyl at 62.8 μg g^{-1}), which is well below levels currently reported to cause adverse effects to plants. Combined benomyl and carbendazim half-lives in soils across treatments were 2−3 months. This study demonstrated that further production and accumulation of DBU in soils after Benlate application or from residual benomyl remaining in the soil are highly unlikely and that persistence of any DBU in soils is likely to be short-lived.

KEYWORDS: ***N,N′*-Dibutylurea (DBU); *n*-butylisocyanate; Benlate; soil; fungicide**

INTRODUCTION

Benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazole carbamate] is the active ingredient in Benlate fungicides (E. I. duPont De Nemours and Co. Inc., Wilmington, DE). DuPont recently discontinued the sale of benomyl after more than 30 years of its use as a fungicide (*1*). From 1897 through March 1992, DuPont sold benomyl as the Benlate 50 DF formulation (50 DF), which was the subject of crop damage claims by growers. One proposed cause of crop damage is *N,N′*-dibutylurea (DBU), a breakdown product of benomyl (**Figure 1**). In aqueous media, benomyl is converted to 2-benzimidazole carbamate (MBC) and *n*-butylisocyanate (BIC). BIC reacts with water to form butylcarbamic acid, which quickly decarboxylates to give *n*-butylamine (BA) and CO_2. DBU forms when BA reacts with the remaining BIC (*2*−*7*). This sequence of reactions has also been shown to occur in sealed ampules containing Benlate formulations with no added water, because sufficient water to hydrolyze BIC is present in the inert ingredients (*4*).

Most of the research related to DBU formation from BIC has focused on formation in solutions or benomyl formulations prior to application to soils and on DBU phytoxicity. In aqueous solutions, DBU formation from BIC is pH dependent (*5*). In solutions with pH <6, only protonated butylamine (BAH^+) (pK_a = 10.8) (*8*) is formed, and further reactions with BIC to DBU are extremely slow. Both DBU and BAH^+ are formed between pH 6 and 11, with DBU being the primary constituent above pH 8. At pH >11, only carbamate is formed. DBU has also been shown to form on plant leaves exposed to BIC vapors emitted from solution followed by adverse effects to ferns and cucumber plants (*3*). When applied as a soil drench, a reduction in root and shoot growth of cucumbers was noted only at DBU rates greater than 94 mg L^{-1} (*9*) and 22.4 kg/ha (9.6 mg/kg) (*10*), respectively. Other toxicity assays for hydrilla, seed germination, and seed emergence for cucumbers, lettuce seedlings, ornamental peppers, and isolated chloroplasts have also shown varying degrees of toxicity to DBU (*4*, *10*−*12*).

No work has been published investigating the potential for DBU to be formed in soils following benomyl application. We hypothesized that in the pH range relevant to most soils (pH between 4 and 8), BA will exist as an organic cation; therefore, it will quickly sorb to soil cation exchange sites when produced from BIC, rendering it unavailable to further react with BIC to form DBU. In the current study, we investigate the potential for DBU to be formed in soil following (1) application of BIC to four different soils at two negative water potentials (0.03

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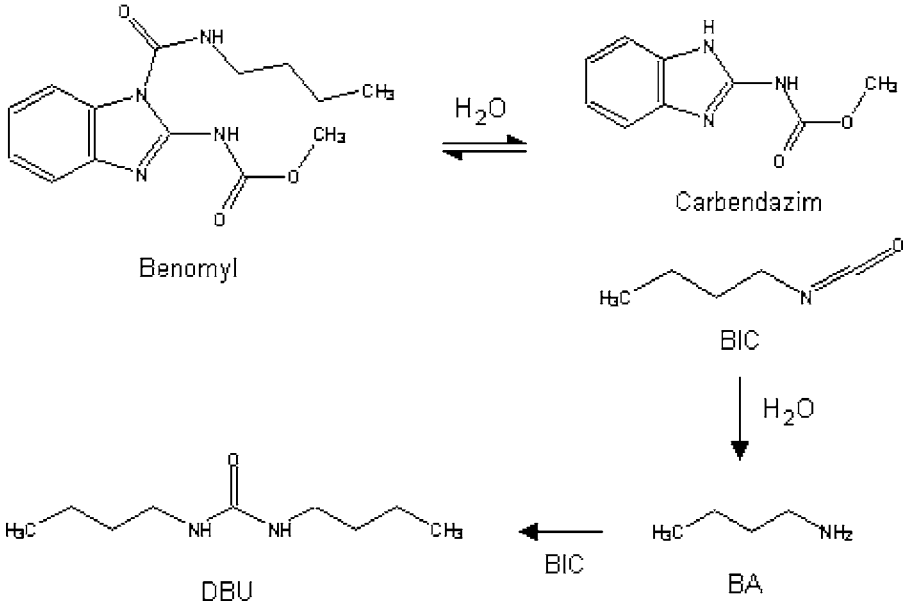

Figure 1. Pathway for benomyl breakdown to carbendazim, *n*-butyl isocyanate (BIC), and butylamine (BA) and combining of BIC and BA to form *N*,*N*′-dibutylurea (DBU).

Table 1. Soil Physical Properties[a]

soil	pH[b]	texture	moisture content[c] (%) at 0.03 MPa	moisture content[c] (%) at 0.1 MPa	clay (%)	OM[d] (%)	CEC[e]	total N (mg kg^{-1})	P (Bray, Olsen) (mg kg^{-1})	site
1AC	6.0	clay loam	0.446	0.332	32.8	4.0	36.7	2164	3, 7	central Costa Rica
2AC	5.8	clay	0.491	0.353	52.8	4.1	43.5	2380	39, 18	central Costa Rica
7CB	6.3	sandy loam	0.793	0.588	6.8	9.1	41.0	5200	11, 4	northern Costa Rica
9RD	7.9	sandy loam	0.226	0.147	16.4	3.7	13.0	1818	115, 158	southern Florida

[a] Determined by MDS Harris Laboratories, Lincoln, NE, under GLP (Good Laboratory Practice). [b] pH at 1:1 g mL^{-1} in water. [c] Percent by dry mass. [d] Organic carbon. [e] Cation exchange capacity in mequiv 100 g^{-1}.

and 0.1 MPa) and (2) application of Benlate 50 DF and 50 SP formulations to two soils at various combinations of negative water potential (0.03 or 0.1 MPa) and temperature (23 or 33 °C).

EXPERIMENTAL METHODS

Materials. Butylisocyanate (BIC) (*butyl*-1-^{14}C-labeled, 23.5 mCi mmol^{-1} specific activity) dissolved in toluene, di-*n*-butylurea (DBU) (*carbonyl*-^{14}C-labeled, 55 mCi mmol^{-1} specific activity) dissolved in ethanol, *n*-butylamine (BA) hydrochloride (*butyl*-1-^{14}C-labeled; 23.5 mCi mmol^{-1} specific activity), and benomyl (*butyl*-1-^{14}C-labeled; specific activity = 20.9 mCi mmol^{-1}) were purchased from NEN Life Science Products (Boston, MA). Benlate 50 SP (soluble powder) and Benlate 50 DF (dry flowable) fungicides were provided by DuPont (Newark, DE) and stored at −80 °C. ScintiSafe liquid scintillation counting cocktail and sodium sulfate (99+%) were purchased from Fisher Scientific (Fair Lawn, NJ). Calcium chloride was of ACS grade and purchased from EM Science (Gibbstown, NJ). Toluene (ACS grade), acetonitrile (HPLC grade), and acetic acid (ACS grade) were purchased from Mallinckrodt (Paris, KY). Aniline was purchased from Sigma Chemical Co. Inc. (St. Louis, MO) with a reported purity of >98% and was further purified by double distillation. Powdered cellulose and dibutylamine (DBA) of ACS grade were purchased from Aldrich (Milwaukee, WI). Combustaid, Carbosorb E, and Permafluor E+ scintillation cocktail were purchased from Perkin-Elmer (Boston, MA). Water was purified by reverse osmosis followed by filtration through a Barnstead Easy Pure LF ultrapure water system.

Soils. Three soils were collected from agricultural fields in Costa Rica, where the soils are somewhat acidic (pH range 5.8−6.3), and one alkaline soil (pH 7.9) was collected from southern Florida. Soils were collected from areas where litigation issues with regard to Benlate use were pending. Although benomyl had been used extensively in these areas, the soils collected for this study were taken from agriculturally relevant sites that had not been treated with benomyl for at least the three years immediately prior to sampling. Each soil sample represents a mix of multiple subsamples taken from the surface (top 3−4 in.) at least 3 ft apart from a 2500-ft^2 sampling area until ∼25 kg of soil was obtained. Moist soils were passed through a 2-mm sieve, mixed well, and stored moist at 4 °C prior to use. Soils were characterized by MDS Harris Laboratories (Lincoln, NE) using the following standard techniques: pH measured from a 1:1 water-to-soil ratio, modified ammonium acetate method for cation exchange capacity (CEC), and organic matter by loss on ignition. Selected soil characteristics including pH, organic carbon (OC) content, soil moisture content for negative soil water potentials of 0.03 and 0.1 MPa, and cation exchange capacity (CEC) are summarized in **Table 1**.

Solutions and Sample Preparation. DBU stock solution was prepared by diluting 5 μL of DBU source material to 10 mL with 45:55 v/v acetonitrile/water. The DBU solution to be added to the soil for assessing recoveries was prepared by a 60-fold dilution in water of the DBU stock. BA stock solution was prepared by dissolving 4 mg of *n*-butylamine hydrochloride in 5 mL of 45:55 v/v acetonitrile/0.1% acetic acid. The BA solution to be added to the soil for assessing recoveries was prepared by a 50-fold dilution in water of the BA stock. Toluene was dried by passing through a bed of anhydrous sodium sulfate (dried at 110 °C for 3 h) and stored in a sealed vial. A BIC solution of ∼240 mg/L to be added to the soil was prepared by diluting BIC source material with toluene. This BIC solution was prepared at a moderately high concentration to minimize the amount of toluene being concomitantly added into the soil with BIC. Soils were fortified with the BIC solution immediately after preparation to avoid reaction of BIC prior to application.

For experiments with benomyl (*butyl*-1-^{14}C-labeled), solutions to be added to the soils were prepared by placing benomyl formulations (∼29 mg for 50 DF or ∼31 mg for 50 SP) into 35-mL glass centrifuge tubes with Teflon-lined lids. Benomyl (*butyl*-1-^{14}C-labeled) (∼1.5 mg) was added to the centrifuge tube followed by 28 mL of water and subjected

to ultrasonication at 20 kHz for 5 min to disperse the solid benomyl. The mixture was then placed on a rotary end-over-end mixer for 30 min prior to application to soils. A carbendazim stock solution of ~100 mg L^{-1} was prepared by dissolving carbendazim in 1:1 v/v acetonitrile/methanol. Analytical standards were prepared by diluting carbendazim stock solution in acetonitrile/water such that the final matrix composition was 70:30 v/v water/acetonitrile. KOH solution (1 M) was prepared daily by boiling water to remove dissolved gases, cooling the water to room temperature in a sealed container, and then adding KOH pellets. The extraction solution for extracting soils after incubation was an aqueous solution containing 0.25 M $CaCl_2$, 0.02 M DBA, and 0.01 M aniline. Ca^{2+} and DBA were added to serve as cation exchange agents for the extraction of BA. Aniline ($pK_a = 4.63$) (*13*) remains unprotonated during extraction and reacts with BIC forming a stable product for chromatographic analysis.

BIC Application. Laboratory studies were used to assess transformation and degradation of BIC using standard soil microbial microcosm techniques (*14*). Soils (~3.8 g of oven-dried mass) were preincubated at 22.7 ± 0.7 °C at the designated water potential in 100-mL wide-mouth jars with Teflon-sealed enclosures for 3 days prior to fortification with BIC. Moist soil microcosms or microcosms with no soil (controls) were fortified with BIC at a rate of 1.2−1.3 μg of BIC g^{-1} of soil using a micropipet (approximately 20 μL or 2 drops of a 240 mg L^{-1} solution), which is equivalent to a 6.8 mol % conversion of benomyl to BIC at the field application drench rate for Benlate [109 lb of active ingredient (ai) $acre^{-1}$]. A 15-mL vial containing 13 mL of 1 M KOH solution was added to each jar (except for day 0 and control samples) to trap $^{14}CO_2$, resulting from the complete mineralization of BIC. The jars were quickly sealed with Teflon-lined closures and incubated at room temperature (22.7 ± 0.7 °C). CO_2 traps were replaced daily prior to extraction of the soil. The traps were assayed using liquid scintillation counting (LSC). Soils were extracted twice sequentially (30 mL each time) while in the microcosm jars. For day 0 and control samples, extraction solution (30 mL) was added prior to fortification with BIC. Extractions were performed on an end-over-end rotary mixer for 20 h followed by centrifugation at 600*g* for 15 min. Aliquots (1 mL) of the clear supernatant were taken for each extract, and LSC analysis was performed. The remaining supernatants from the two extracts were combined and analyzed by both LSC and reverse-phase liquid chromatography (RPLC) to determine percent recovery. After extraction, the soils were oxidized to determine nonextractable radiolabel.

Benomyl Application. To assess the potential formation of DBU from Benlate 50 DF and 50 SP fungicides, formulations prepared with *butyl*-1-^{14}C-labeled benomyl were incubated in 2AC and 9RD soils at 0.03 and 0.1 MPa water potentials and at 23 and 33 °C. Soils (4.5 g of dry mass) were preincubated at a specific temperature and water potential in 100-mL wide-mouth jars with Teflon-sealed enclosures for 3 days prior to fortification as in the BIC fate experiments. Benomyl formulations were added to the moist soil microcosms at a rate of 60−97 μg of benomyl g^{-1} of soil. This application rate is equivalent to 111−180% of the drench rate for Benlate fungicide application (109 lb of ai $acre^{-1}$). Soils and nonsoil control samples were fortified in triplicate with 0.5 mL of a benomyl solution. For day zero and nonsoil control samples, methanol (20 mL) was added before benomyl additions and the soils were extracted as described for BIC except soils were extracted twice sequentially with methanol (20 mL). Extracts were stored at 4 °C in the dark until sample preparation and analysis could be performed (<1 week). Loss of benomyl as carbendazim (benomyl is converted to carbendazim in the methanol extraction) was tracked with RPLC-UV, formation of [^{14}C]DBU was tracked by RPLC radioassay detection, production of [^{14}C]CO_2 was tracked by LSC in the KOH traps, and postextraction soil-bound ^{14}C was assessed by combustion.

DBU, BA, and Benomyl Controls for Recovery. Air-dried soils (3.7−4.5 g of oven-dried mass) were added to 100-mL wide-mouth jars with Teflon-lined enclosures. DBU, BA, or benomyl solutions (0.5 mL) were added to soils and no-soil control samples in duplicate. Samples were extracted ~1 h after application using the same procedure as described for the BIC and benomyl fate experiments.

For incubations with benomyl formulation, soil was extracted with methanol to recover benomyl and/or carbendazim residues and any remaining DBU immediately after application of [^{14}C]benomyl-laced 50 DF or 50 SP and subsequent incubations. Methanol extraction quantitatively converts benomyl to carbendazim. Using this method, it is not possible to distinguish between benomyl and carbendazim in the soil. Therefore, both benomyl and carbendazim residues are quantified as carbendazim in the extract.

Solid Phase Extraction (SPE). To improve detection of DBU in the BIC incubations, SPE and concentration were performed on extracts prior to RPLC analysis using SPE tubes (SDB-L, 500 mg and 3 mL, Phenomenex, Torrance, CA). SPE tubes were preconditioned with 3 mL of acetonitrile followed by 3 mL of water. A 25-mL aliquot of extract was added at a rate of <2 mL min^{-1} followed by washing of the cartridge with 2 mL of water. DBU was then eluted with 2 mL of acetonitrile and the eluent assayed by RPLC. The fractions obtained were analyzed by LSC to determine percent recovery.

For the incubations with [^{14}C]benomyl-enriched formulations, the concentration of radiolabel in the sample extracts was determined by LSC. An aliquot (10 mL) of the sample extract was then taken, evaporated to dryness under reduced pressure with a gentle stream of nitrogen, and reconstituted in 3 mL of 30:70 v/v acetonitrile/water using sonication to ensure complete dissolution of analytes. The sample was filtered through a 0.45-μm PTFE filter and analyzed by RPLC. The concentration of radiolabel after RPLC sample preparation was determined by LSC.

RPLC Analysis. The RPLC system consisted of the Shimadzu 10Avp series with system controller (SCL-10Avp), two pumps (LC10-ATvp), vacuum degasser (DGU-14A), autoinjector (SIL-10A), and variable-wavelength UV detector (SPD-10Avp) at 210 nm. A flow-through radioassay detector (Raytest Ramona, Raytest USA, Inc., Wilmington, NC) equipped with a CaF scintillator flow cell was placed in line after the UV detector. Column temperature was maintained at 40 °C using a Waters temperature controller. Separations were performed using gradient elution on a reverse-phase C_{18} column (Luna 150 mm L × 4.6 mm i.d., 5-μm particle size, Phenomenex) with a precolumn guard (C_{18}-ODS, 4.0 mm L × 3.0 mm i.d., Phenomenex) at a flow rate of 1 mL min^{-1}. Solvent A was 10 mM sodium acetate buffer (pH 5.0). Solvent B was 40:60 v/v 25 mM sodium acetate buffer (pH 5.0)/acetonitrile. For BA and BIC analysis, the mobile phase composition was 90:10 v/v A/B for the first 4 min followed by an increase of solvent B to 100% from 4 to 6 min, holding at 100% from 6 to 18 min, and then a decrease to 10% (starting composition) from 18 to 19 min. Retention times for BA and BIC were 3.3 and 15 min, respectively. Two small unidentified peaks were also observed at approximately 4.8 and 6.8 min in two soil incubations under these conditions. For DBU analysis, the initial mobile phase composition was the same (90:10 v/v A/B) followed by an increase to 100% B from 0 to 12 min, holding at 100% from 12 to 25 min, and then a decrease to 10% (starting composition) from 25 to 26 min. A 5-min equilibration time at 90:10 v/v A/B was allowed between injections. Retention times for DBU and BIC under these conditions were 11.5 and 18.5 min, respectively.

For analysis of solutions from the benomyl fate study, solvent A was 15:85 v/v 50 mM sodium phosphate (pH 7.0)/water, and solvent B was 15:25:60 v/v/v 50 mM sodium phosphate (pH 7.0)/water/acetonitrile. Three different reverse-phase columns (150 mm L × 4.6 mm i.d., 5-μm particle size) with corresponding guard columns were used over the course of the study: Luna C_{18} column (Phenomenex), Suplecosil LC-AZB+ (Supelco, Bellefonte, PA), and Supelcosil LC-18 (Supelco). Initial mobile phase compositions were 10−35% B followed by gradient slopes between 2.4 and 3.6% B min^{-1} depending on the column used. All gradients had a final mobile phase composition of 60% B followed by re-equilibration at initial conditions for 8−10 min. Injection volumes were 200 μL for all analyses.

LSC. The LSC system was a Packard 1600TR (Perkin-Elmer, Boston, MA). Aliquots (1 mL) were mixed with 15 mL of ScintiSafe 30% in 20-mL scintillation vials. Vials were kept undisturbed in the dark for 24 h prior to LSC.

Postextraction Oxidation. Soils were analyzed for ^{14}C content by combustion using a Packard model 307 sample oxidizer (Perkin Elmer, Boston, MA). Soil (0.2−0.5 g of dry weight equivalent) was weighed into a paper cup, mixed with ~200 mg of powdered cellulose, placed

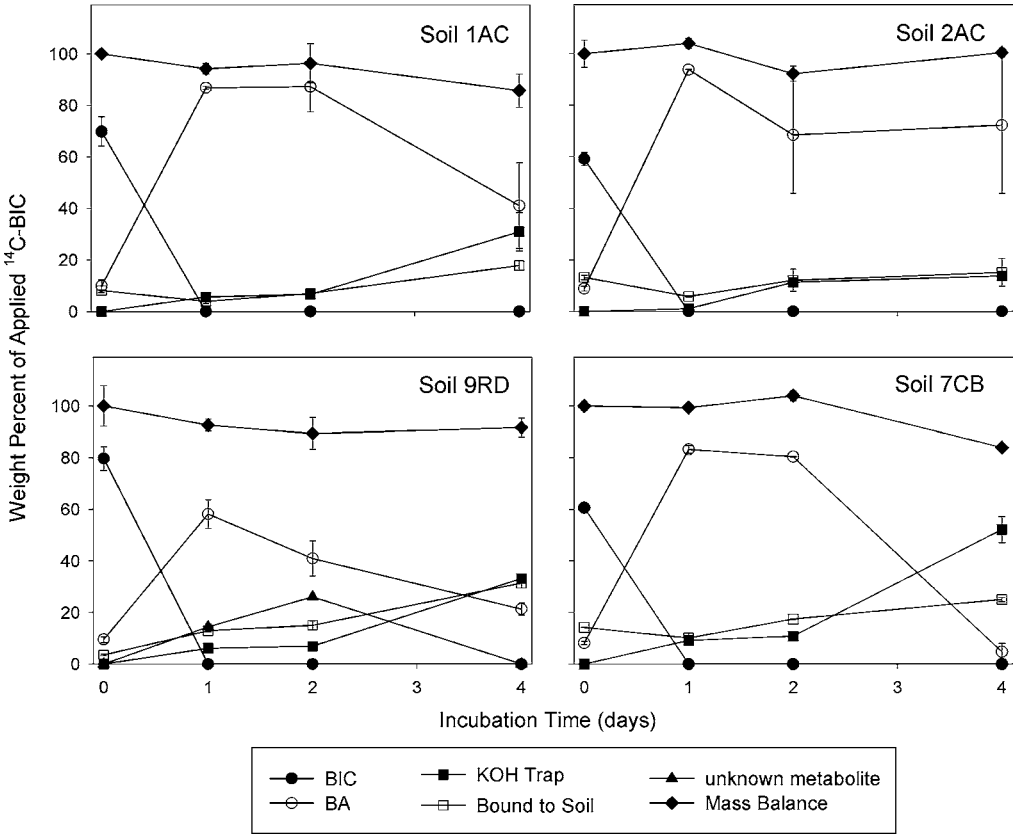

Figure 2. Fate of ^{14}C-labeled BIC and metabolites in soils treated with 1.3 μg of BIC g^{-1} and incubated at 0.03-MPa negative water potential and 23 °C. Error bars represent standard deviations ($n = 3$).

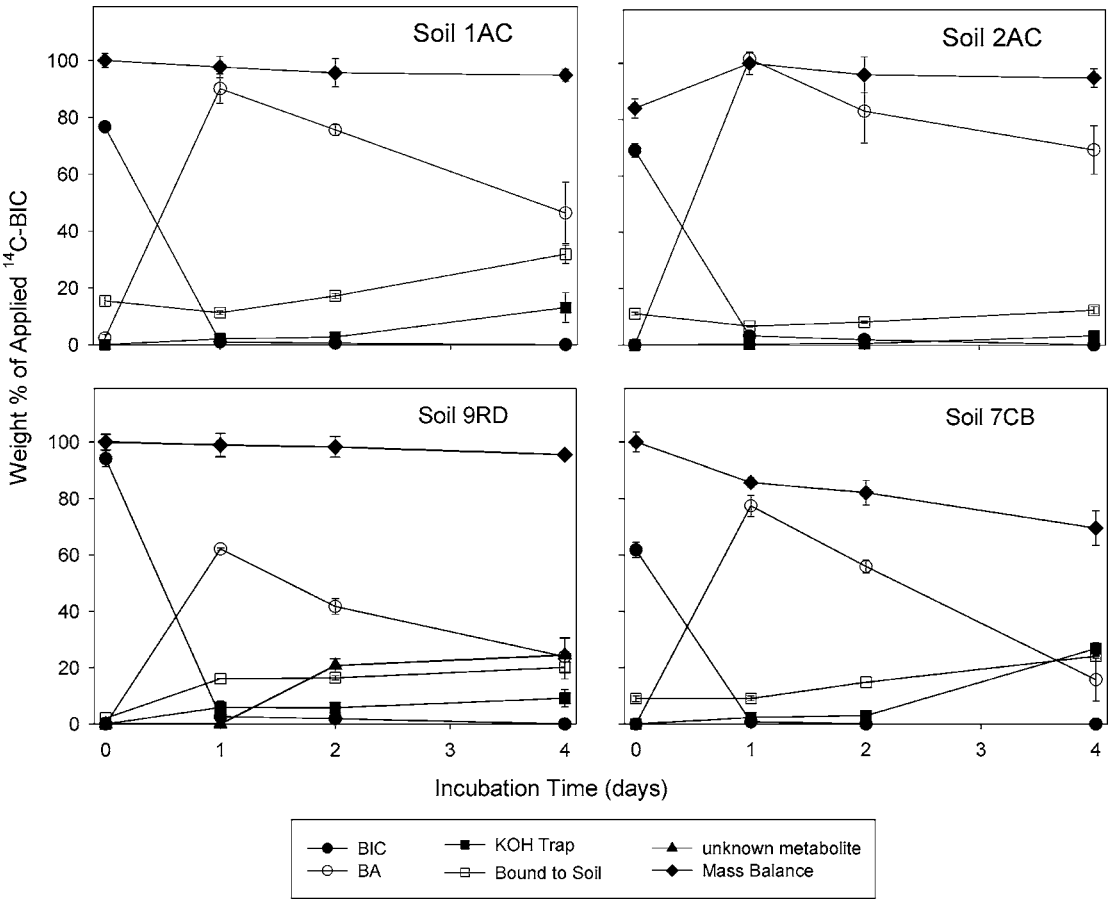

Figure 3. Fate of ^{14}C-labeled BIC and metabolites in soils treated with 1.2 μg of BIC g^{-1} and incubated at 0.1-MPa negative water potential and 23 °C. Error bars represent standard deviations ($n = 3$).

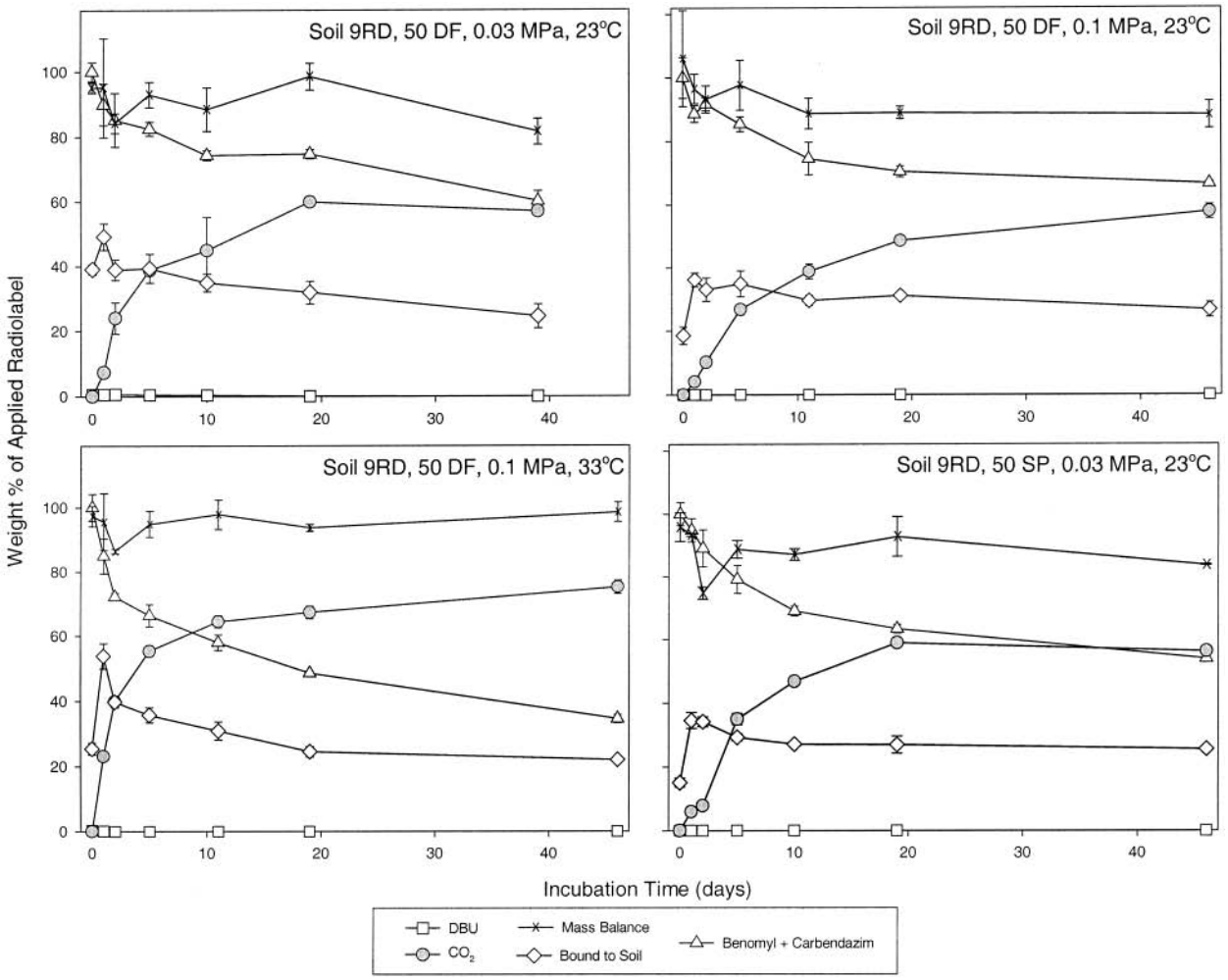

Figure 4. Fate of [*butyl*-1-^{14}C]benomyl-enriched Benlate 50 DF or 50 SP applied at an application rate between 63 and 86 μg g^{-1} in soil 9RD incubated at 0.03- or 0.1-MPa negative water potential and 23 or 33 °C. Error bars represent standard deviations ($n = 3$).

in the instrument, and combusted for 3 min. Combustaid (0.1 mL) was added to moist soil samples prior to combustion to facilitate complete oxidation. Carbosorb E (10 mL) reagent was used to trap $^{14}CO_2$, and Permafluor E+ scintillation cocktail (10 mL) was added to the sample. Samples were left in the dark for 24 h before ^{14}C determination by LSC. Quenching was compensated by using a quench curve constructed with Carbosorb E and Permafluor E+. Radioactivity originating from residual extracting solvent after the second extraction was subtracted.

Barium Chloride Precipitation. A barium chloride precipitation assay was used to ensure that the KOH traps did not reach saturation and to determine the fraction of ^{14}C-label present as CO_2. Aliquots of the solutions from each of the CO_2 traps (3 mL) were transferred into 15-mL polypropylene centrifuge tubes and treated with 6 mL of 1.5 M $BaCl_2$ on day 1 for the BIC experiments and with 0.6 mL for subsequent sampling and all sampling of the Benlate experiments. The tubes were placed on a rotary end-over-end mixer for 1 h. After mixing, the tubes were centrifuged at 600*g* for 15 min. Parallel samples were processed except that water was added instead of $BaCl_2$. Samples (1 mL) were assayed by LSC to determine the fraction of radiolabel precipitated. All of the radiolabel that precipitated was assumed to be CO_2.

RESULTS AND DISCUSSION

BIC Application. The fate of [^{14}C]BIC at 23 °C was monitored over a 4-day period in all four soils at negative water potentials of 0.03 and 0.1 MPa. Shown in **Figures 2** and **3** relative to the initial [^{14}C]BIC applied are the weight percents (wt %) of the ^{14}C-labeled BIC, BA, and an unidentified metabolite extracted, the ^{14}C from soil oxidation after extraction, and the ^{14}C assayed in the KOH traps as a function of time for 0.03 and 0.1 MPa, respectively. Also shown is the total ^{14}C mass balance estimated from LSC of the combined soil extracts, which includes all of the extractable ^{14}C, $^{14}CO_2$ measured in the KOH traps, and the postextraction oxidation of the soil. BIC rapidly declined after application and was no longer detected by day 4. By day 2, most of the BIC was found as BA, followed generally by a decline in BA concentration with time. The percent of ^{14}C evolved measured in the postextraction oxidation and in the KOH traps increased over the 4-day period. Formation of DBU from BIC was not deteced in any of the soils incubated at the higher water content (0.03 MPa). Recoveries of DBU and BIC of control samples were 97.2 ± 2.9 and 89.3 ± 2.5%, respectively, with a method detection limit for DBU of 1.2 μg of DBU kg^{-1} of soil. Under dryer conditions (0.1 MPa), small amounts of DBU at a maximum of 3.6 μg/kg (<0.3% of applied BIC) were detected on day 4 in soils 1AC and 2AC. This level of DBU is far below both the lowest level reported to show a

Table 2. Summary of BIC Mineralization Rates Incubated at 23 °C and 0.03- and 0.1-MPa Water Potentials in Four Soils (Standard Errors Are Given in Parentheses)

	rate (day^{-1})	
soil	0.03 MPa	0.1 MPa
1AC	–0.101 (0.093)	–0.034 (0.035)
2AC	–0.084 (0.100)	–0.032 (0.021)
7CB	–0.109 (0.082)	–0.029 (0.026)
9RD	–0.181 (0.158)	–0.081 (0.084)

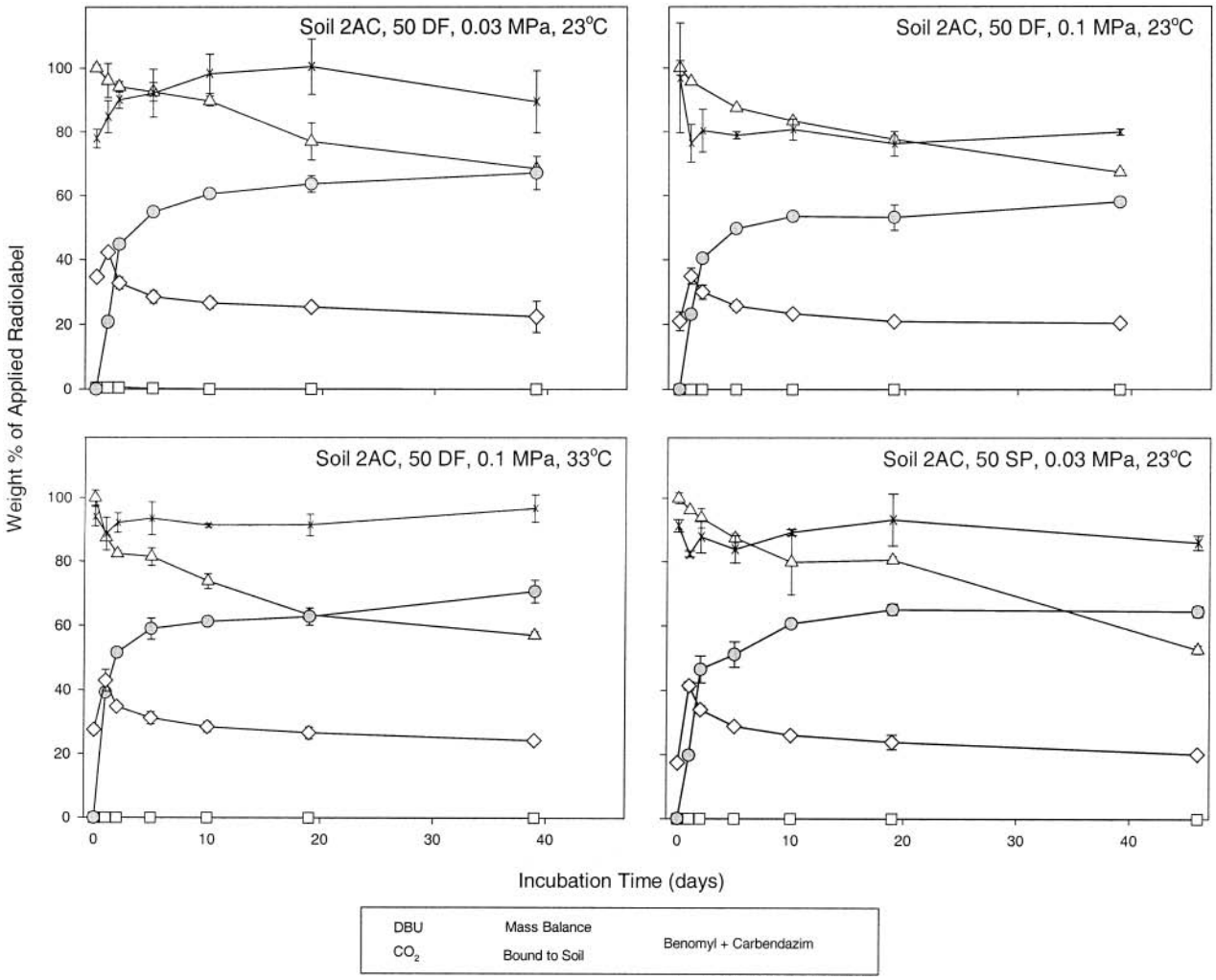

Figure 5. Fate of [*butyl*-1-^{14}C]benomyl-enriched Benlate 50 DF or 50 SP applied at an application rate between 63 and 86 μg g^{-1} in soil 2AC incubated at 0.03- or 0.1-MPa negative water potential and 23 or 33 °C. Error bars represent standard error deviations ($n = 3$).

negative impact on plants (*3*, *9*−*11*) and noted to affect photosynthetic electron transport in isolated chloroplasts (*12*). By day 4, no BIC remained in any of the soils; therefore, no further formation of DBU could occur after this time. Some of the radiolabel (12−32%) was not extractable after 96 h, which suggests incorporation (covalent bonding) of metabolites within soil organic matter.

In three of the four soils studied, >80% of the BIC was converted to BA after 1 day, with the remainder of the radiolabel recovered as $^{14}CO_2$ or in the postextraction oxidation (**Figures 2** and **3**). The BA content of soil 9RD was lower at ∼60% with some of the radiolabel (∼20% maximum) appearing as an unidentified peak (6.8 min) in the RPLC trace. In the same RPLC traces, a second unidentified peak at 4.8 min was observed but was ∼3 times smaller than the later unknown peak and was difficult to accurately quantify. At a 0.03-MPa water potential, both unidentified metabolites were present on days 1 and 2 but completely dissipated by day 4. However, at 0.1 MPa these metabolites do not appear until day 2 and are still present on day 4. The slower formation and dissipation of the unidentified compounds under dry (0.1 MPa) conditions suggest that they result from microbial degradation of BIC.

Several attempts were made to identify the unknown metabolites using LC/APCI-MS (Thermo-Finnegan LCQ) with full scans between *m*/*z* 70 and 127 and between *m*/*z* 132 and 200 (large background peaks existed between *m*/*z* 128 and 131). For day 1 and 2 samples from soil 9RD incubations, only one peak (*m*/*z* 115) could be differentiated from the background noise and was not present in other samples. We were not able to hypothesize a chemical structure that matched the *m*/*z* 115 peak. Given the low concentrations of the metabolites (low parts per billion levels for the larger 6.8-min peak) coupled to the noise in the low molecular weight range of interest, a further assessment using MS/MS was not possible.

A significant fraction (9−52%) of the radiolabel applied as BIC was found as CO_2 after 4 days of incubation. $BaCl_2$ precipitation indicated that ∼70% of the radiolabel appearing in the trap during the first day of incubation and 20% of the radiolabel appearing in the trap during the second day of incubation were not CO_2. KOH solutions from the trap after $BaCl_2$ precipitation and neutralization with acetic acid were analyzed using HPLC as described previously for DBU. The radiolabel that was not CO_2 eluted at the same retention time as DBU; volatilized BIC captured in the KOH trap was converted to DBU. The latter was confirmed with controls where BIC was spiked directly into KOH solutions. Of the total radiolabel measured in the traps over the entire 4-day period, >84% was CO_2 resulting from the complete mineralization of BIC. First-order mineralization rates are summarized in **Table 2**. Overall, mineralization rates were 2−5 times faster under the more optimal conditions for microbial activity (0.03 MPa), and mineralization was fastest for soil 7CB, which had an OC and total nitrogen content 2 times higher than any of the other soils. In all cases, even with direct application to soil of BIC,

Table 3. Summary of Degradation and Mineralization Rates and Half-Lives of [*butyl*-1-^{14}C]Benomyl-Enriched Benlate 50 DF and 50 SP Fungicides and Extent of Degradation at the End of the Incubation Period

					$^{14}CO_2$					combined benomyl + carbendazim		
soil	form	MPa	T (°C)	time[a] (days)	%[b]	rate[c] (SE)[d] (day^{-1})	first-order (days)	$t_{1/2}$ (days)	visual 50%[f] (days)	%[g]	rate (SE) (day^{-1})	$t_{1/2}$ (days)
2AC[h]	DF	0.03	23	39	67	0.110 (0.021)	10	8.2	3.6	68.5	0.011 (0.001)	72
2AC	DF	0.1	23	39	58	0.095 (0.020)	10	10.4	5.5	67.3	0.011 (0.001)	73
2AC	DF	0.1	33	46	71	0.213 (0.047)	5	39.2	1.9	57	0.015 (0.003)	66
2AC	SP	0.03	23	46	65	0.070 (0.015)	19	14.6	4.2	52.6	0.014 (0.001)	52
9RD[h]	DF	0.3	23	39	57	0.054 (0.006)	19	15.3	11.7	60.1	0.015 (0.002)	64
9RD	DF	0.1	23	46	58	0.074 (0.014)	19	37.8	23.3	66.5	0.027 (0.004)	92
9RD	DF	0.03	33	46	75	0.039 (0.003)	19	27.2	4.0	34.5	0.011 (0.002)	34
9RD	SP	0.03	23	46	59	0.052 (0.005)	19	14.4	12.4	53.9	0.016 (0.003)	55

[a] Total incubation time of experiment. [b] Total $^{14}CO_2$ evolved at the end of incubation time. [c] $^{14}CO_2$ evolution curves were not first-order at later times; thus, psuedo-first-order rates were estimated from the ln-linear portion of the curve as detailed in the next column. [d] Standard errors. [e] Cutoff day for first-order fit. [f] Visual observation of when 50% of the ^{14}C label had evolved. [g] Benomyl remaining at the end of incubation time. [h] DBU formation was observed.

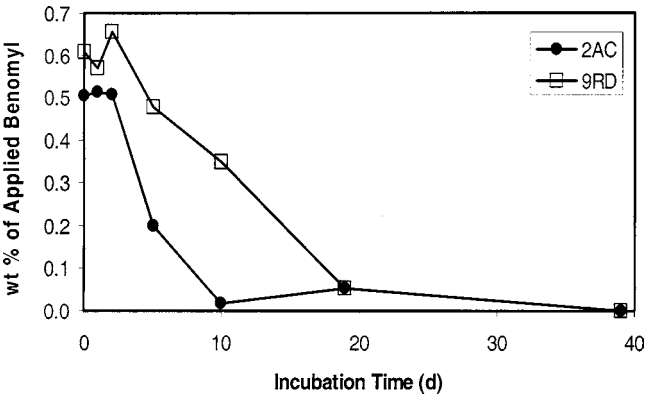

Figure 6. DBU formation as a weight percent of applied benomyl in soils 2AC and 9RD incubated with [*butyl*-1-^{14}C]benomyl-enriched Benlate 50 DF at 0.03-MPa negative water potential and 23 °C.

a required precursor for DBU formation, most of the BIC is converted to BA with little or no DBU formation.

Benlate 50 DF and 50 SP Application. To assess DBU formation in a more realistic scenario, the fate of benomyl in 50 DF and 50 SP formulations enriched with [*butyl*-1-^{14}C]-benomyl was measured in soils 2AC and 9RD at 0.03- and 0.1-MPa water potentials and at 23 or 33 °C. The selected subset of two soils (2AC and 9RD) represented the range in pH, clay content, and sampling location of the four soils in this study. Extraction efficiency for benomyl and carbendazim varied from 77 to 96%. The fraction of combined benomyl and carbendazim remaining was normalized to unity at day 0. Recovery of DBU using the SPE procedure was 93.2 ± 2.9%. RPLC analysis of BA and BIC was performed prior to SPE.

Representative dissipation curves of benomyl and carbendazim were combined, and subsequent metabolites are shown in **Figures 4** and **5** for soils 2AC and 9RD, respectively, along with a total mass balance for the ^{14}C label. DBU formation was not observed in any combination of soil type, water potential, temperature, and formulation type except for incubation with [*butyl*-1-^{14}C]benomyl-enriched 50 DF at a 0.03-MPa water potential and 23 °C (**Figure 6**). However, maximum DBU concentrations observed were only 0.51% on days 1 and 2 in soil 2AC and 0.65% on day 2 in soil 9RD, and in both cases this is followed by a subsequent decrease in DBU over time. The last sampling day in which DBU was observed in either of these two incubations was day 19 at 0.05%, followed by the absence of DBU on the next sampling day (39 days).

Mineralization and combined benomyl−carbendazim degradation rates are summarized in **Table 3** along with the half-lives calculated by assuming first-order kinetics and [^{14}C]-benomyl remaining and $^{14}CO_2$ evolved at the end of the incubation period. $^{14}CO_2$ evolution (mineralization) was not completely described by a first-order process; therefore, mineralization rates are estimated using only the portion of the curve that appeared to be of first-order. The last day on which first-order behavior was apparent, and therefore the last day included in the first-order fits, is noted in the table along with the observed half-life based on the day when 50% of the ^{14}C label had evolved as $^{14}CO_2$. Mineralization rates were dependent on soil type, temperature, and water content. Soil 2AC exhibited faster mineralization than the 9RD soil. In both soils, mineralization rates were higher at 33 °C. Mineralization rates were slightly slower under the dryer conditions (0.1 MPa), especially for soil 9RD, but the effect on combined benomyl and carbendazim dissipation rates was smaller. By the end of the incubation period, between 57 and 75% of the applied radiolabel had been collected as CO_2. Although mineralization appeared to be slower near the end of the incubation period, a longer incubation would likely have resulted in additional mineralization. Rates of benomyl and carbendazim degradation exhibited trends with soil type and treatment conditions similar to those for mineralization. In soils 2AC and 9RD across treatments, half-lives for benomyl−carbendazim combined were 2−3 months, which is within the range of half-lives (1−6 months) observed for various soil conditions (*15*−*17*). Fungicide formulation type (50 DF or 50 SP) had little effect on either mineralization or benomyl degradation rates. At the end of the incubation period, between 34 and 69% of the applied benomyl remained.

ENVIRONMENTAL SIGNIFICANCE

DBU is a metabolite that can be formed in the production of Benlate fungicides or during improper storage. Although a low level of DBU was observed to form in a few of the treatments, the amount of DBU was very small, with a maximum concentration observed of 0.41 μg g^{-1} (0.65 wt % of applied benomyl at 62.8 μg g^{-1}), which is well below levels currently reported to cause adverse effects to plants. Any DBU formed completely disappeared after a few weeks. Recent work by Lee et al. (*18*) shows that DBU degrades rapidly in soils in both the presence and absence of the formulated material. This study

demonstrates that further production and accumulation of DBU in soils after Benlate application or from residual benomyl remaining in the soil are highly unlikely and that persistence of any DBU in soils is likely to be short-lived.

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