N,N'-Dibutylurea from n-Butyl Isocyanate, a Degradation Product of Benomyl. 1. Formation in Benlate Formulations and on Plants†

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n-Butyl isocyanate, an elimination product of benomyl, the active ingredient in Benlate formulations, reacts with water to give the symmetrical *N,N'*-dibutylurea and the salt of butylamine. Reaction is first order with a rate constant between 7.3×10^{-4} and 8.4×10^{-4} s^{-1}. When moist Boston fern and cucumber plants are exposed to *n*-butyl isocyanate, *N,N'*-dibutylurea is formed on or in the plants' leaves. This compound, along with *N*-butylformamide and *N*-butylacetamide, has been found in both dry flowable and wettable powder formulations of Benlate fungicide.

INTRODUCTION

Benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate] is a broad spectrum fungicide used on many types of food and ornamental plants (Fuchs et al., 1972; Lyda and Burnett, 1970; Delp and Klopping, 1968). It has been shown that benomyl eliminates *n*-butyl isocyanate (BIC) in both water and organic solvents to produce carbendazim [methyl 2-benzimidazolecarbamate (MBC); Calmon and Sayag, 1976]. The rate of elimination is greatly dependent upon the nature of the solvent (Chiba and Cherniak, 1978). In water, the isocyanate undergoes a first-order reaction with the production of butylamine, a result of the rapid decarboxylation of butylcarbamic acid (Calmon and Sayag, 1976). It has long been known that isocyanates react with water to ultimately produce the substituted ureas (Arnold et al., 1957). When benomyl is dissolved in water, the *N,N*-dibutylurea is formed (Axness and Fleeker, 1979).

Recent studies have shown that BIC vapors are found in the head space above a stirred suspension of the Benlate formulation of benomyl, ostensibly as a result of elimination from benomyl (Tang et al., 1992).

Other recent studies have shown that *N,N'*-dibutylurea (DBU), a potential product from the reaction of BIC and water, is phytotoxic to plants when applied at relatively low rates as a drench to their roots (Shilling et al., 1994).

The research described here was intended to determine whether or not *N,N'*-dibutylurea could be formed in typical Benlate formulations of benomyl fungicide, as a result of such butyl isocyanate elimination from benomyl and subsequent reaction with residual water in the formulations, and on moist plant surfaces. We also intended to identify any other products that may have arisen from the reaction of butyl isocyanate and water and which might subsequently prove to be phytotoxic to plants.

EXPERIMENTAL PROCEDURES

Instrumentation. Mass spectral data were collected on a Hewlett-Packard Model 5989A (MS Engine), equipped with a Model 5890 II gas chromatograph and a Model 7673A autosampler. Both electron impact and chemical ionization (methane) spectra were taken. All chromatography was performed on a PTE-5 capillary column, 30 m by 0.25 mm i.d., 0.25-μm film thickness. The following temperature program was used throughout: initial temperature, 100 °C for 2.0 min, ramp at 30 °C/min to a final temperature of 275 °C, hold for 3 min, cool to 100 °C, and equilibrate for 3.0 min. Injector temperature was at 240 °C and was operated in the splitless mode.

Gas chromatographic analyses were performed on a Hewlett-Packard 5890 capillary gas chromatograph equipped with a Model 7671A autosampler and with the column and program as described above. The detector was a Detector Engineering Technologies Model TID-2-H_2/air/nitrogen/phosphorus with a TID-2 thermionic source operated at a bead current of 3000 mA (Walnut Creek, CA). Detector temperature was at 350 °C. All samples were freshly prepared and sealed in amber GC autosampler vials.

Nuclear magnetic resonance (NMR) data were taken on two separate instruments. Carbon-13 data were taken at 75.45 MHz on a Nicolet Model NT300 spectrometer, with a magnet having a field strength of 70 kG. Proton data were taken on a Varian Unity at 500 MHz. Both instruments were capable of taking proton-coupled or -decoupled spectra. Some ^{13}C experiments were performed in the coupled mode as a means of determining the number of protons attached to each carbon.

Photolysis of *n*-butyl isocyanate and water vapors was conducted in a Rayonet Model RMR-500 minireactor, equipped with four 15-W lamps, Model RPR-3000Å.

Materials. All reagents were 99%+ in purity. DBU was obtained from K&K Laboratories, ICN Biochemical Co.; butylamine, butyl isocyanate, and butylurea came from Aldrich Chemical Co. *N*-Butylacetamide (NBAc) came from Eastman Kodak Co. Benomyl was supplied by the DuPont Co. and analyzed to be 99.15% pure. Ethyl acetate, 2-propanol, and water were of HPLC grade from Fisher Scientific. NMR spectra were taken in deuterated dimethyl sulfoxide or in water containing 10% by volume D_2O, obtained from Aldrich Chemical Co. and Sigma Chemical Co., respectively. Both butyl isocyanate and butylamine are fairly volatile and toxic to animals and, consequently, should be handled with caution.

Mature Boston ferns, with frond lengths of approximately 60 cm, were obtained locally; immature (60–80 mm in height) cucumber plants (*Cucumis sativus* L.) were seeded and propagated by the authors. The ferns were placed in seed starter soil (A. H. Hoffman, Inc.) containing peat moss, vermiculite, and limestone. Exposure of the cucumbers to BIC was done in the coarse sand in which they were propagated.

A total of 37 boxes or bags of Benlate formulation were examined by gas chromatography for DBU. Of these, only 13 were freshly opened in our laboratory or the laboratory of the Division of Plant Industry, Florida Department of Agriculture

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Table 1. Summary of DBU and NBAC Levels Found in Formulations[a]

formulation lab ID	lot	date of manufacture	DBU %	DBU SE	NBAC μg/g	NBAC SE
DFA	BATCH813	June 15, 1989	2.53[d]	0.36	71.7	1.8
DFB	216870326	Dec 1987	1.49	0.12	52.8	1.1
DFC	U0401900267P2	April 1990	0.65	0.06	56.8	2.6
DFD[g]	U071590-687	July 1990	0.36	0.03	45.3	4.2
DFE[g]	_09-90 0220 p02	March 1990	0.13	0.03	84.6	3.8
DFF[g]	_17-90_370 p02	May 1990	0.17	0.03	102.0	4.2
DFG[g]	_0220 p 02	March 1990	0.15[c]	0.04	101.6	5.1
DF5065	U83189	Aug 1989	3.49	0.55	249.2	9.6
DF5070	U062490-616	June 6, 1990	1.62	0.13	210.8	4.2
DF5071	(from grower)	[b]	2.23	0.22	192.8	3.7
DF5073	U073190-738	July 2, 1990	0.44	0.04	117.5	6.8
DF5077	U080790-753	Aug 5, 1990	0.78	0.07	126.2	12.4
DF5080	U061490-579	June 7, 1990	1.18	0.05	196.8	7.8
DF5081	U072490-715	July 5, 1990	1.01	0.07	159.4	5.0
DF5082	286	Jan 21, 1989	3.71	0.35	381.5	10.0
WPA[g]	F042391E	April 1991	0.45	0.06	0.0	0.0
WPB[g]	EP42_61MS1	[b]	3.44	0.41	37.9	2.4
WPC	_E70519B_	[b]	8.85[c]	0.20	28.8	0.9
WPD	F0524916	May 1991	0.64	0.06	0.0	0.0
WPE	F60505H	prior to 1989	7.52	1.11	191.9	7.4
DF1	277	Oct 1988	7.05	1.04	455.3	24.7
DF3[g]	6-U062090-596	June 1, 1990	0.33[d]	0.02	70.3	2.5
DF4[g]	2498	Aug 1988	6.71[d]	0.63	219.2[f]	
DF5	210602-B	[b]	5.74	0.53	305.8	10.2
DF6[g]	12	[b]	0.59	0.03	143.8	3.3
DF7	R 19-7655	[b]	6.17	1.32	206.7	7.9
DF8[g]	U062490-615	June 5, 1990	0.36	0.01	71.2	1.0
DF9[g]	U072490-717	July 4, 1990	0.30[d]	0.02	63.3	6.0
DF10	-	[b]	6.80[d]	0.71	297.2	13.4
DF11	U9989-0190P5	Sept 1989	4.85	0.44	271.3	13.0
DF12	BDF187	[b]	0.8	0.08	113.2	4.1
DF13	0318880B727	[b]	1.85	0.15	206.2	9.2
DF16	-	[b]	5.10[c]	0.03	87.6	2.8
DF17[g]	Batch813	June 15, 1989	2.22[c]	0.10	269.0	11.3
DF18[g]	B_2 48	[b]	7.43[c]	0.68	556.3	9.2
DFds	-	[b]	0.50[c]	0.03	[e]	
WPds	-	[b]	0.38[c]	0.02	[e]	
benomyl 99%	-		0.21[c]	0.01	[e]	

[a] DF, dry flowable formulation; WP, wettable powder formulation; benomyl, pestinal technical grade benomyl used as control; DBU analyzed in quadruplicate and NBAC analyzed in duplicate, except where noted. [b] No manufacturer date available. [c] Analyzed in duplicate. [d] Analyzed six times. [e] Not analyzed. [f] Analyzed once. [g] Opened by our personnel.

and Consumer Services; the remaining were opened and stored by others under a variety of conditions. Those opened at the University or the Division of Plant Industry were kept at laboratory temperatures, approximately 25 °C, and humidities, approximately 60–70%. These formulations ranged in their dates of manufacture from December 1987 to July 1990.

Extractions of formulations and kinetic experiments were done in 20-mL glass liquid scintillation vials with Teflon-lined screw caps. Sonications were accomplished in a Branson Model B-220 sonicator, 125 W, with 4 cm of water in the reservoir.

Boston ferns and immature cucumber plants were exposed to BIC vapors in 10-gal glass aquaria with glass lids. Vapor containment by the lids was accomplished with high-vacuum silicon stopcock grease. A 10-mL volumetric flask was used to contain the BIC liquid in the aquaria.

Fern leaves were extracted in 500-mL Mason jars using an Omni-Mixer Model 17105.

Methods. *Analysis of Formulations.* Exactly 50 mg of powdered Benlate formulation was placed in a 20-mL scintillation vial, to which 10 mL of ethyl acetate or 2-propanol was added. The vial was capped and sonicated for 5 min. Particulates were removed by filtration using a Swinney adapter containing a 0.45-μm pore size filter. The supernatant was diluted 1:50 for analysis by GC. Analytical curves of DBU, 0.05–50 μg/mL, were prepared daily. Duplicate injections of each laboratory replicate, usually six (see Table 1), were made and the average was taken. Similar amounts of analytical grade benomyl were extracted with ethyl acetate and 2-propanol to determine whether DBU could be formed by a direct conversion.

Kinetics of n-Butyl Isocyanate in Water. Kinetics of DBU formation in water were determined using both gas chromatography and proton NMR spectroscopy.

In the gas chromatography experiments, 0.44 mmol of BIC was added to 50 mL of HPLC grade water at 25 °C in a capped Erlenmeyer flask. At about 5-min intervals, 1-mL aliquots were removed and added to a 20-mL scintillation vial containing 5 mL of ethyl acetate. The vial was capped and shaken for 2 min. After approximately 1 min, 1 mL of this solution was added to 4 mL of ethyl acetate and placed in amber autosampler vials. Injections of 2 μL of this solution were made on the gas chromatograph.

In the proton NMR experiments, 0.027 mmol (3 μL) of BIC was added to 0.7 mL of distilled H_2O/D_2O (9:1), D_2O, or deuterated dimethyl sulfoxide (DMSO) contained in a 5-mm NMR tube at 30 °C. The proton spectrum of the deuteriochloride salt of butylamine was taken by adding 5 μL of butylamine to 10 μL of deuterium chloride in 700 μL of D_2O. The pH was adjusted to 9.57 with deuterated sodium hydroxide in D_2O.

In the ^{13}C experiments the total sample volume was 3.2 mL in a 12-mm tube containing 0.123 mmol (13.7 μL) of BIC in 2.9 mL of HPLC grade water and 0.3 mL of D_2O. Similar concentrations of BIC were examined in 3.2 mL of DMSO.

Comparisons were made with authentic DBU in D_2O, in slightly more dilute solutions (0.027 mmol in 1.5 mL). Spectra were also taken of BIC in deuterated DMSO and of butylamine in both solvent systems at 0.027 mmoles in 0.7 mL.

Exposure of Ferns and Cucumber Plants to n-Butyl Isocyanate. Fern frond clusters containing six or seven fronds each, were shaken free of soil and placed in 600-mL beakers, to which seed starting soil was then added to a volume of 400 mL. The soil was gently tamped down, and exactly 200 mL of deionized water was added, giving a soil moisture content of near capacity. Three beakers were placed at one end of each of four aquaria. Each set of fern fronds was gently misted with deionized water until run off from the leaves began. At the opposite end of two of the four aquaria a capped 10-mL volumetric flask containing 200 μL of BIC (1.8 mmol; 0.09 mmol per frond) was placed. All aquaria were placed in fume hoods equipped with two 60-W fluorescent tubes placed about 60 cm above the plants. The volumetric flasks containing the BIC were then uncapped, the lids placed on the aquaria, and the lights turned on. Temperature was maintained at approximately 25 °C. Observations were taken every 24 h.

A similar experiment was done with immature cucumber plants, except that 10 pots containing 2 plants per pot were placed in each aquarium. Only one control aquarium was prepared for this experiment.

For each experiment, the plants were harvested after 72 h. The beakers and pots were removed from the aquaria and prepared for extraction. Each fern frond was clipped about 6 cm above the soil and cut into 2-cm segments, placed into Ziploc freezer bags, and held at −20 °C until extraction. Each cucumber plant was clipped at the sand surface, placed into Ziploc freezer bags, and similarly stored.

Fern leaves, in 25-g portions (triplicate), were placed in 500-mL Mason jars together with 50 mL of ethyl acetate and blended on the Omni-Mixer at low speed for 5 min. The slurry was decanted into a Büchner funnel containing 1 in. of sodium sulfate; 1.5 mL of each extract was placed in amber autosampler vials for analysis by gas chromatography.

Cucumber plants, in 5-g portions (triplicate), were placed in 50-mL conical centrifuge tubes and chopped fine with a sharp-ended spatula. Ten milliliters of ethyl acetate was added to each tube, and the tube was capped and shaken for 5 min on a flat bed shaker. The solvent was decanted, in 5-mL portions, into a 10-mL glass syringe containing 3 mL of sodium sulfate and forced through the syringe with the plunger into a 20-mL scintillation vial. Aliquots were taken for gas chromatography analysis.

Mass Spectral Data Acquisition. Both electron impact and chemical ionization data were acquired upon injection of ethyl acetate solutions (10 ng/μL) of DBU and *N*-butylacetamide onto the capillary column, using the temperature program described

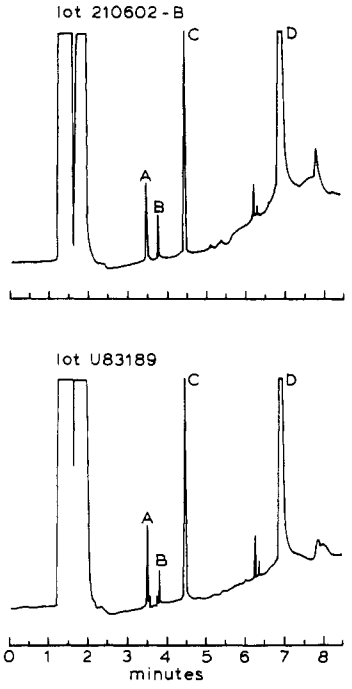

Figure 1. Two dry flowable formulations of Benlate containing *N*-butylformamide (peak A), *N*-butylacetamide (peak B), *N*-butyl-2-propylcarbamate (peak C), and DBU (peak D). See text for details.

above. Mass spectra were also acquired for the fern leaf and cucumber plant extracts, as well as for selected formulations.

RESULTS

Analysis of Formulations. Various concentrations of DBU found in the 37 Benlate formulations by extraction with ethyl acetate are given in Table 1. Weight percentages of DBU ranged from a low of 0.13% to a high of 8.85%. *N*-Butylacetamide concentrations, as determined by extraction with 2-propanol, were found in 32 of the formulations, with none found in 2 of them, and 5 were not quantitated. Also tentatively identified in several of the formulations was *N*-butylformamide, which was always present when *N*-butylacetamide was found, usually in larger amounts. Representative GC chromatograms are shown in Figure 1. Peak C, *N*-butyl-2-propylcarbamate, results from the reaction between *n*-butyl isocyanate and the extraction solvent, 2-propanol; benomyl eliminates *n*-butyl isocyanate quite readily in this solvent. A small amount of DBU was found in the analytical grade benomyl used as a control, less than 0.25% by weight. An experiment that exposed benomyl to 2-propanol was done to determine whether significant amounts of BIC were released during the 5-min extraction process, which might react with residual water to form DBU. This experiment was done at benomyl levels that simulated what is found in typical formulations. We found no measurable DBU formation during the benomyl extractions.

Kinetics of Butyl Isocyanate in Water. The kinetics curve for the generation of DBU in water is shown in Figure 2. It fits a first-order rate equation, with an r^2 of 0.9974 and a rate constant, k_1, of 8.4×10^{-4} s^{-1}, which is consistent with the pseudo-first-order kinetics which are to be expected here. The half-life was calculated to be 13.8 min. No other peaks appeared on the chromatogram other than DBU, which appeared at 6.95 min, and solvent, since the other compound formed, the butylamine salt, does not partition from the water. A summary of retention data

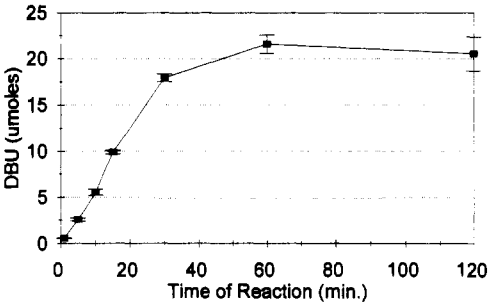

Figure 2. *n*-Butyl isocyanate in water, 8.8×10^{-3} M, 25 °C. DBU was determined by gas chromatography after extraction with ethyl acetate. See text for details.

Table 2. Retention Times for Standards

compound	retention time (min)	notes: minor peaks
DBU	6.95	single peak
butylurea	5.46	major peak (others <5%): 1.95
butylamine	3.66	many peaks: 2.42, 2.72, 3.86, 4.36, 5.61
butyl isocyanate	1.95	small peaks: 6.04, 8.71, 10.16
N-butylmethylcarbamate	3.62	small shoulder: 6.83
N-butylacetamide	3.87	single well-resolved: MW115

for various compounds anticipated and found in this study, under the temperature program that was used, appears in Table 2.

When both the proton and carbon nuclear magnetic resonance spectra were examined, it was clear that a second material was being formed in addition to DBU. Comparison with the proton spectrum of the butylamine salt formed from deuterium chloride addition to butylamine showed its identity to be that compound. It appeared to form at a somewhat faster rate than DBU, having a first-order rate constant of 9.2×10^{-4} s^{-1}, compared to 7.3×10^{-4} s^{-1} for DBU. Comparison of the butyl group intensities showed that the ratio of butylamine salt to DBU was approximately 1.3.

The following proton spectra were recorded.

DBU in deuterated dimethyl sulfoxide: δ 0.85 (t, 6H, $2CH_3$), 1.23 (m, 4H, $2CH_2$), 1.32 (m, 4H, $2CH_2$) 2.50 (solvent), 2.95 (t, 4H, $2CH_2$ next to NH), 3.35 (s, trace H_2O), δ 5.68 (br s, 2H, 2NH).

DBU in water (10% D_2O): δ 0.82 (t, 6H, $2CH_3$), 1.24 (h, 4H, $2CH_2$ next to CH_3), 1.37 (p, 4H, $2CH_2$), 3.02 (q, 4H, $2CH_2$ next to NH), 4.65 (s, solvent and NH in exchange equilibrium), 5.79 (br s, very small, NH).

Butylamine in deuterated dimethyl sulfoxide: δ 0.85 (t, 3H, CH_3), 1.28 (m, 4H, $2CH_2$), 2.05 (v br, NH_2), 2.50 (m, CH_2, next to NH_2, overlapping solvent resonance).

Butylamine in deuterium oxide: δ 0.95 (t, 3H, CH_3), 1.40 (h, 2H, CH_2 next to CH_3), 1.50 (p, 2H, CH_2), 2.69 (t, 2H, CH_2 next to NH_2), 4.80 (s, solvent and NH_2 in exchange equilibrium).

Butylamine salt (deuteriochloride) in deuterium oxide (pH 9.57): δ 0.92 (t), 1.38 (s), 1.60 (p), 2.98.

BIC in deuterated dimethyl sulfoxide: δ 0.88 (t, 3H, CH_3), 1.33 (h, 2H, CH_2 next to CH_3), 1.52 (p, 2H, CH_2), 3.34 (t, 2H, CH_2 next to —N=C=O).

BIC in water, taken immediately after mixing: δ 0.83 (t, CH_3), 1.31 (h, CH_2 next to CH_3), 1.52 (p, CH_2), 3.27 (t, CH_2 next to —N=C=O).

Unidentified reaction product in water, taken 1 h after addition of BIC (pH 9.17): δ 0.93 (t, CH_3), 1.39 (h, CH_2 next to CH_3), 1.64 (p, CH_2), 3.00 (m, CH_2 next to a

functional group). The peak at δ 3.00 is approximately a triplet but resembles more the AA′ part of an AA′XX′ pattern.

The following carbon-13 proton-decoupld spectra were taken.

DBU in water–3% D_2O: δ 16.1 ($2CH_3$), 22.2 ($2CH_2$, adjacent to CH_3 groups), 34.3 (middle CH_2 of each butyl group), 42.7 (CH_2 groups adjacent to secondary NH), 163.8 (amide carbon). All signals were sharp; no line splitting was seen. Proton-coupled spectra, also acquired, confirmed the assignments of methyl, methylene, and carbonyl residues.

BIC in deuterated dimethyl sulfoxide: δ 12.9 (CH_3), 19.1 (CH_2 adjacent to CH_3 group), 32.6 (middle CH_2 group), 42.1 (CH_2 group adjacent to N), 121.5 (isocyanate C). All signals were sharp; no line splitting was seen.

BIC in water, DBU set: δ 15.9 (CH_3); 22.2 (CH_2 adjacent to CH_3 group), 34.2 (middle CH_2 group), 42.6 (CH_2 group adjacent to N), 163.7 (amide carbon).

BIC in water, "unknown" set: δ 15.6 (CH_3), 21.8 (CH_2 adjacent to CH_3 group), 31.7 (middle CH_2 group), 42.3 (CH_2 group adjacent to N), 163.1 (amide carbon). Note: signal heights of "unknown" set are 25–40% larger than those of DBU.

Analysis of Fern and Cucumber Plants. Although these experiments were designed only to provide an answer to the question of whether DBU could be formed in or on plants as a result of BIC exposure, it became obvious, within 24 h, that both sets of plants were experiencing phytoxicity. After 24 h, the ferns began to show formation of brown or "bronze" spots on the leaves. After 72 h, they were totally brown and fell from the stems when handled. No discoloration was noted for the untreated (control) plants.

Cucumber plants exposed to BIC vapors behaved somewhat differently. A spotted browning of the cotyledons was first noted at 24 h, followed by a browning of the guttation water exuding from them. By 72 h, this browning to the mature leaves had increased but had not covered the entire leaves, as had occurred for the ferns. After 72 h, the mature leaves exhibited intravenal yellowing and a yellowing around the edges. No discoloration or guttation was noted for the untreated (control) plants.

Easily measurable levels of DBU were found in both treated fern and cucumber leaves (6.95 min). A typical chromatogram of a cucumber extract is shown in Figure 3. No DBU was found in the control plants. The levels of DBU found on these plants are shown in Table 3.

Mass Spectra of Standards, Plants, and Formulations. Both electron impact and chemical ionization mass spectra are presented in Table 4. Ion intensities, relative to the base peak, are in parentheses.

DISCUSSION

Of the 37 different Benlate formulations analyzed here, more than half (21) contained levels of DBU greater than 1.0% by weight. Fourteen of these 37 formulations were selected at random and were not associated with perceived plant damage. Thirteen of the 37 were unopened until laboratory or greenhouse studies were conducted; those that were opened for such study were stored in a humidity- and temperature-controlled laboratory facility after opening, not exceeding 70% relative humidity and 27 °C. Those formulations that had been opened by users ranged widely in their storage conditions, probably being stored in farm chemical sheds or warehouses that are typical in Florida, although this was not verified.

It was observed that small amounts of *N*-butylacetamide were formed in the injector of the gas chromatograph when

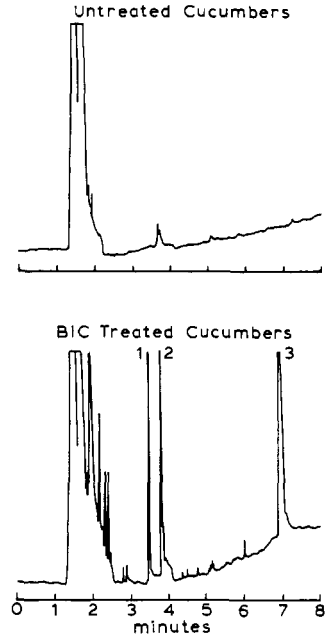

Figure 3. Chromatograms of ethyl acetate extract of cucumber leaves, 72 h after exposure to BIC; NP detector. Peak 3, DBU. See text for details.

Table 3. DBU Analysis in Ferns and Cucumbers

treatment	sample	*n*	DBU (μg/g)	range DBU (μg/g)
		In Ferns		
control	BEN42C	6	0.00	
BIC	BEN42T1	3	0.73	0.58–0.88
BIC	BEN42T2	3	2.87	2.80–3.00
control	BEN44C	6	0.00	
BIC	BEN44T1	3	0.80	0.80–0.80
BIC	BEN44T2	3	4.23	1.00–10.60
		In Cucumbers		
control	BEN125C	2	0.00	
BIC	BEN125T1	3	6.55	2.23–12.18
BIC	BEN125T2	3	8.73	1.82–17.45

Table 4. Mass Spectra (EI and CI[a])

compound	ions [*m/z* (relative abundance)]
dibutylurea	44 (100), 41 (34), 57 (25), 172 (24), 74 (24), 43 (12)
N-butylacetamide	43 (100), 44 (58), 72 (53), 73 (44), 41 (20), 60 (17)
N-butylformamide EI	58 (100), 72 (60), 100 (15)
N-butylformamide CI	102 (100), 130 (8), 142 (8)

[a] Methane reagent.

ethyl acetate solutions of *n*-butylamine were injected. Consequently, formulations were reanalyzed using 2-propanol as the extraction solvent; results of these analyses eliminated the possibility that *N*-butylacetamide was formed from the presence of residual *n*-butylamine reacting with the extraction solvent. Two of the formulations analyzed, both wettable powders, as seen in Table 1, were free from *N*-butylacetamide. Consequently, it is unlikely that the other samples listed as having levels of that chemical were contaminated during laboratory workup. In addition, an injector temperature study was done, where the responses of both *N*-butylacetamide standard and that found in formulations were measured by keeping the same column temperature program but varying the injector in increments from 240 °C down to 100 °C. No differences were seen between standards and formulations for a given amount of *N*-butylacetamide when peak heights and peak shapes were inspected. This indicates that the compound

is probably not a result of on-column pyrolysis of one or more precursors.

Upon examination of Table 1 for *N*-butylacetamide levels in the formulations, it is apparent that the ratios of *N*-butylacetamide to DBU amounts are far less for the wettable powders than for the dry flowable formulations. How these differences occurred is unknown.

The data presented here show that not only does DBU appear in a significant number of formulations sampled, it has the potential of being formed on leaf surfaces as a result of contact with BIC and the subsequent water reaction. Its effects on cucumbers following single drenches has recently been demonstrated, causing chlorosis, stunting, and chloroplast damage (Shilling et al., 1994). Our observations with the aquaria-incubated plants are consistent with this recent finding, even though our plant exposure was via BIC vapors and at levels high enough such that other similarly volatile organics might have caused similar symptoms. Whether the plant phytotoxicity that we observed was due to BIC, DBU, or the butylamine salt was not addressed by these experiments. Transfer of DBU to the plant leaf surfaces by volatilization from the volumetric flasks within the aquaria seems highly unlikely, considering its 82 °C melting point. Peaks 1 and 2 in the chromatogram of the treated plants are *N*-butylformamide and *N*-butylacetamide, respectively, resulting from the pyrolysis of ethyl acetate and *n*-butylamine on the GC injector.

The chemical produced from benomyl by the elimination of butyl isocyanate, carbendazim, would of necessity be produced in equimolar amounts and would not be expected to be phytotoxic, since it is the active ingredient in a number of presently marketed fungicides. Analyses for it were not performed in these experiments.

DBU and the butylamine salt are the only products detected in the NMR kinetic study of BIC in water. This result indicates that the reaction rates for decarboxylation of the carbamic acid to butylamine and for the reaction between butylamine and another molecule of BIC are extremely fast. Kinetic experiments on BIC added to a solution of butylamine in D_2O revealed only trace amounts of each after 1.5 min. Thus, butylamine appears to be rapidly scavenged by available BIC. No *N*-butylacetamide or *N*-butylformamide was observed in this aqueous-based reaction.

Further work is in progress to determine the fate of DBU in soil and long-term plant studies at low concentrations of DBU in soil.

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