Effect of Temperature and Humidity on the Formation of Dibutylurea in Benlate Fungicide†

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Benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate] is the active ingredient in DuPont Benlate fungicides. The formation of *N*,*N*′-dibutylurea (DBU), a phytotoxic degradation product of benomyl, in Benlate formulations was evaluated by analyzing Benlate samples maintained under simulated storage conditions and assessing the effects of temperature and humidity on sample moisture content, benomyl degradation, and the rate of DBU formation. Benomyl degraded during storage by the elimination of *n*-butylisocyanate (BIC) to form methyl 2-benzimidazole carbamate (MBC; carbendazim). Liberated BIC could then proceed to react with water to form DBU (first-order rate constant of 8.4×10^{-4} s $^{-1}$). The degradation of benomyl and subsequent formation of DBU were dependent on the temperature and highly dependent on the humidity of the storage environment. At the lower humidity storage conditions the rates of DBU formation were significantly higher in the dry flowable (DF) formulation than in the wettable powder (WP) formulation. The initial moisture content of Benlate DF samples was higher than those of Benlate WP samples, although the Benlate WP samples absorbed more moisture upon incubation. These results may yield insight on the appearance of high levels of DBU found in some boxes and bags of Benlate DF and Benlate WP formulations.

Keywords: *Benlate; benomyl; fungicide; N,N-dibutylurea; formulation; carbendazim*

INTRODUCTION

Benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate] is a broad spectrum fungicide registered for use on many types of food and ornamental plants (Fuchs et al., 1972; Lyda and Burnett, 1970; Delp and Klopping, 1968). Benomyl was first introduced by E. I. DuPont de Nemours, Inc., in 1967 and registered with the U.S. Department of Agriculture in 1969 as a wettable powder (WP) formulation under the trade name DuPont 1991. It was also sold under the product name Benlate WP and Tersan 1991. In 1987, DuPont introduced a dry flowable (DF) product, Benlate DF, and gradually phased out production of the WP formulation. Benlate has been implicated in a number of cases of crop damage and, because of concerns over phytotoxicity, DF production was halted. Although benomyl products are registered by several companies, the WP formulation is the only product currently labeled by DuPont for use in the United States.

A number of boxes and bags of Benlate formulations of various ages and sources were analyzed for *N*,*N*′-dibutylurea (DBU) by Moye et al. (1994). Of the 35 different containers analyzed, nearly half (17) contained levels of DBU >2.0 wt %. In every Benlate sample analyzed, DBU was found at concentrations >1000 μg/g, with some bags containing as much as 80000 μg/g. Of these 35 containers, 14 were selected at random and were not associated with perceived plant damage.

Aragaki et al. (1994) showed that cucumbers exposed to WP or DF Benlate formulations indirectly in glass chambers with only a gas exchange between the plants and formulations showed significant growth retardation. The authors speculated that *n*-butylisocyanate (BIC) liberated from the benomyl degradation was responsible for the phytotoxicity. They demonstrated the presence of BIC vapors in the headspace of their growth chambers. Although BIC was a potential source of direct phytotoxicity, the authors failed to account for potential reaction products of BIC released from the Benlate suspensions. Due to the reactive nature of isocyanates and the large amount of water in the chamber atmosphere needed to support plant growth, it seems likely that DBU would also be present in the plant tissue. Moye et al. (1994) reported that DBU was detected on leaf surfaces of plants exposed to BIC vapors alone.

Shilling et al. (1994) have shown that DBU is phytotoxic to plants as a drench to the roots, when applied at rates found in Benlate field samples. The results show a dose-dependent decrease in cucumber root and shoot weight with increasing dose of DBU. Statistically significant decreases compared to untreated controls were seen with application rates of 2.8 kg/ha DBU applied as a single drench to 7-day-old plants when harvested at 21 days old. Symptomology of DBU phytotoxicity included chlorosis, stunting, and chloroplast damage similar to that seen for diuron, a phenyl-substituted urea herbicide. Photosynthetic O_2 evolution as well as the rate of peak to terminal chlorophyll *a* fluorescence in treated leaf isolates confirmed the

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similar mode of action between DBU and other substituted ureas (Querns et al., 1998).

Similar phytotoxic results have been shown when Benlate formulations containing DBU were applied as a root drench to ornamental peppers (S. Walker, personal communication, 1998). Benlate DF injury indices (marginal leaf scorch and chlorosis) were highly correlated to the DBU content of the Benlate formulation (Shilling et al., 1994). When Benlate formulations, which give a low injury score, were fortified with DBU to the levels of Benlate formulations that gave high injury scores, they produced injury which was qualitatively and quantitatively similar to the high injury level of native DBU (Tolson and Moye, 1996). Furthermore, DBU was found to rapidly translocate in the plant system and concentrate in the leaf tissue. These results indicate that DBU and not a second co-degradate was responsible for the observed phytotoxicity.

If DBU arises from the reaction of BIC eliminated from benomyl and residual water (Moye et al., 1994), then the rate at which it is formed in Benlate formulations could be expected to depend on available water, temperature, and ingredients in the formulation other than benomyl. Tang and Song (1996) have shown that DBU is formed spontaneously in Benlate DF formulations stored under elevated temperatures, but they did not examine the effect of controlled humidity. The temperature and storage conditions commonly found in pesticide storage sheds may induce the decomposition of Benlate, resulting in the evolution of DBU. This study was designed to investigate the role of various combined temperature and humidity conditions on the stability of Benlate and the formation of DBU from Benlate WP and DF formulations.

MATERIALS AND METHODS

Materials. All reagents were 99%+ in purity. DBU was obtained from K & K Laboratories, ICN Biochemical Co.; butylamine and butyl isocyanate (BIC) came from Aldrich Chemical Co. Benomyl, *99.15% purity*, was supplied by the DuPont Co. Extracting solvents were of Optima grade and water was of HPLC grade, from Fisher Scientific. Salts used for humidity control were purchased from Aldrich at the highest purity available. Starch (powdered redried A) was supplied as a gift from A. E. Staley Manufacturing Co. Karl−Fisher Reagent was obtained from Fisher Scientific as a single stabilized solution. Benlate formulations were obtained from other state agencies, purchased directly from a local supplier, or collected from growers. Upon receipt at the Food and Environmental Toxicology Laboratory, a laboratory ID was assigned, and the contents of opened bags were transferred to sealed high-density polyethylene containers and stored in a temperature- and humidity-controlled environment not exceeding 60% relative humidity and 27 °C. Those formulations that had been opened by users ranged widely in their prior storage conditions, probably being stored in farm chemical sheds or warehouses that are typical in Florida, although this was not verified.

Instrumentation. Gas chromatographic analyses were performed on a Hewlett-Packard 5890 capillary gas chromatograph equipped with a model 7871A autosampler. 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 set at 350 °C. All samples were made fresh and sealed in amber GC autosampler vials. The column was a Supelco PTE-5, 30 m × 0.25 mm i.d., 0.25 μm film thickness. Carrier gas was nitrogen at 3 mL/min. The injection port temperature was 240 °C, splitless, and the detector temperature was 350 °C. The column temperature program was as follows; initial temperature, 175 °C; hold for 2.0 min; ramp to 230 °C at 20 °C/min; equilibrate at 175 °C for 3.0 min.

High-performance liquid chromatography (HPLC) of benomyl (as STB; 3-butyl-2,4-dioxo-*s*-triazino[1,2-*a*]benzimidazole) was done in the reverse phase mode, with solvent delivery by a Waters model 6000A pump, a Rheodyne model 7125 injector fitted with a 10 μL injection loop, a Perkin-Elmer model LC-75 spectrophotometric detector, a Perkin-Elmer LCI-100 integrator/recorder, and a DuPont Zorbax ODS column, 4.6 mm i.d. × 25 cm. The mobile phase consisted of 45% acetonitrile, 40% water, and 15% buffer A at a flow rate of 0.7 mL/min. Buffer A was prepared according to the method of Chiba and Singh (1988) by mixing 3 parts of a 0.067 M solution of Na_2HPO_4 with 2 parts of a 0.067 M solution of KH_2PO_4 with adjustment of the pH to 6.50 with dilute H_3PO_4. Buffer B, used for sample preparation, was prepared from 1.2 M solutions of Na_2PO_4 and KH_2PO_4, by mixing them in 3 to 2 portions as well.

Mass spectral data of prepared 3-butyl-2,4-dioxo-*s*-triazino-[1,2-*a*]benzimidazole (STB) were taken with a Hewlett-Packard model 5989A (MS Engine), using a Hewlett-Packard series 1050 solvent delivery system and a model 59980B interface. A DuPont Zorbax ODS column, 4.6 mm i.d. × 25 cm, was developed with 45% acetonitrile, 40% water, and 15% buffer A (see below) at a flow rate of 0.7 mL/min.

Temperature control of the incubated Benlate formulations was done in a Percival model 35L incubator. Humidity inside sealed desiccators was monitored with a Fisher Scientific model 661 humidity gauge.

DBU Formation Kinetics. To determine whether DBU could be produced during storage of Benlate formulations, 50 mg portions of WP or DF Benlate formulations were placed in scintillation vials. The opened scintillation vials were stored in a clean sealed desiccator (Wheaton) where the desiccant was replaced with saturated solutions of either K_2SO_4, NaCl, or $MgCl_2$. The headspace above the saturated salt solutions has been shown to maintain a stable humidity environment throughout a wide temperature range (Winston and Bates, 1976). Saturated K_2SO_4, NaCl, and $MgCl_2$ solutions providing humidities of 96, 75, and 32%, respectively, were maintained at either 37 or 23 °C. Triplicate preweighed Benlate formulations were removed after 7, 14, 28, and 56 days and analyzed for DBU. Additionally, nonincubated controls, preweighed, capped, and stored at ambient conditions, were analyzed at each time point.

DBU content was assessed by extraction of the formulation in each vial with 10 mL of 2-propanol. The vials were capped and sonicated for 5 min to ensure complete disruption of particulates and thorough extraction. To each vial an internal standard (trifluralin) was added to achieve a final concentration of 1.45 μg/mL. Samples were allowed to settle for 1 h, and then a 200 μL aliquot was diluted 50-fold for a final formulation concentration of 100 μg/mL. Quantitation was performed on the gas chromatograph by either comparison of DBU peak height to an external standard curve or by the ratio of peak heights of the internal standard to DBU and knowledge of the relative response factor for these compounds.

Benomyl and MBC Concentrations. The benomyl present in the Benlate formulations was determined by HPLC as the stable STB after alkaline derivitization of the parent benomyl.

Because STB is not available commercially, an analytical standard was prepared from technical grade benomyl (DuPont) using a modification of the method described by Singh and Chiba (1985). Approximately 1 g of benomyl was dissolved in 100 mL of 0.1 N NaOH by sonication in a bath sonicator for 5 min. The solution was then allowed to stand for 30 min with intermittent swirling every 5 min to mix. The STB formed by the alkaline treatment was precipitated by adding 1 N HCl until the pH dropped to 1.0. The precipitate was vacuum filtered through a Whatman No. 1 filter paper and rinsed with 3 × 10 mL of 1 N HCl and then 3 × 10 mL of H_2O to remove any MBC residues. The crystals were dried under vacuum in a sealed desiccator overnight. The purity, as determined by LC/MS analysis, was >98%.

Table 1. Moisture Determinations in Benlate Samples

formulation	% moisture	% DBU
WPD	0.81	0.64
WPA	0.85	0.45
WPE	1.20	7.52
DFH	5.05	9.23
DFH9	2.53	0.38
DFF	4.71	0.17
DFG	4.22	0.15
DFUS1	3.65	0.96
DFUS2	3.64	5.74
redried starch	6.18	0
Incubated Samples		
iWPA	36.3	N/A[a]
iWPE	39.2	N/A
iDFG	29.2	N/A
iDFUS2	27.2	N/A
i-redried starch	28.5	N/A

[a] N/A, postincubation DBU quantitiations were not performed.

Moisture Determinations; Water Uptake. Representative Benlate boxes and bags were tested for moisture content using the Karl−Fischer reaction. This method is based on the reduction of iodine by sulfur dioxide in the presence of water. Duplicate 0.5 g samples of Benlate formulations were weighed into tared screw-capped 50 mL centrifuge tubes. Methanol (50 mL) was added, and the tubes were sonicated for 10 min to disrupt the Benlate particles. After sonication, the tubes were centrifuged for 15 min at 500*g* to pellet the insoluble Benlate material. The contents were decanted into an Eylemyer flask and titrated with Karl−Fisher reagent until the appearance of a tawny brown end point, which persisted for at least 1 min. The volume of titrant used was recorded to 0.1 mL, and the strength of the titrating solution was calculated by the titration of standard methanol/water-containing solutions. A control methanol blank was also titrated, and the methanol moisture value was subtracted from the sample value.

Triplicate 1 g portions of both WP and DF Benlate samples containing both high and low levels of DBU were incubated in a controlled 37 °C desiccator maintained at 75% RH above a saturated NaCl solution. Additionally, soluble starch was included as a control because starch is a major inert ingredient in Benlate. Samples were weighed after 7 and 28 days and weights recorded to the nearest 0.05 g. Portions of these samples were analyzed for moisture content, DBU content, and MBC/STB ratios. See Tang and Song (1998) for a similar study without humidity control.

Statistics. The quantitations of DBU and STB were performed in triplicate (unless otherwise indicated) and quantified by comparisons to external standards run on the same day. Replications are noted for the other studies in the appropriate methods section.

In the heat and humidity study the DBU levels were determined for each time point, after which a regression model was used to estimate the slope and variance for that treatment combination. The slopes generated were subsequently used to compare the effects of the various treatments. All statistical analyses were performed using Statistical Analysis System (SAS) for the personal computer (SAS Institute, Cary, NC). The level of statistical significance was taken as $p < 0.05$ (Helwig and Council, 1985).

RESULTS

Moisture Determinations; Water Uptake. Water uptake, expressed in weight gain, was measurably higher for the WP formulations compared to the DF, with an average 72% increase at 75% relative humidity (RH) after 28 days of incubation, compared to only 32% for the DF.

Moisture determinations for both sets of samples before and after incubation at 75% RH for 28 days are given in Table 1. Highest initial moisture was observed for a DF sample (DFH; 5.05%) compared to the highest WP sample (WPE; 1.2%). DBU content did not seem to influence moisture uptake, but rather formulation type did. WP samples averaged 38.4% increase in moisture upon incubation, whereas DF samples averaged only 28.3%. Water uptake was independent of DBU concentrations for both. Starch alone had water uptake similar to that of the DF samples.

Table 2. Percentages of Benomyl, MBC, and DBU in Various Benlate Formulations before and after Incubation at Controlled Humidity and Temperature

formulation	benomyl (as STB) (%)	MBC (%)	MBC + benomyl (total)	DBU (%)	mol of isocyanate recovered[a] (%)
WPA	50.4	4.4	54.8	0.45	96.8
WPD	45.3	6.8	51.4	0.64	87.9
WPE	0.9	50.5	52.1	7.52	30.1
DF1	1.4	52.4	53.8	7.05	29.2
DFH	0.9	49.0	50.0	9.23	36.5
DFF	49.4	6.0	55.3	0.17	93.8
DFUS2	19.6	30.4	50.0	5.74	58.6
DFH9	50.9	3.2	54.2	0.38	97.5
DF5065	21.7	25.0	46.8	3.49	54.1
DF18	1.1	49.9	50.9	7.43	30.1
DFG	47.6	5.2	52.8	0.15	90.4
DFUS1	43.3	7.7	51.0	0.96	85.3
DF10	1.9	50.1	52.0	6.80	29.2
Incubated Samples					
iWPA	11.3	17.1	28.4	N/A[b]	N/A
iWPE	0.8	28.3	29.1	N/A	N/A
iDFUS2	0.9	38.3	39.2	N/A	N/A
iDFG	20.5	19.3	39.8	N/A	N/A

[a] Represents butylcarbamoyl moeity recovered in DBU and intact benomyl compared to what could theoretically be recovered from original unincubated formulation. [b] N/A, no data available.

Table 3. Formation Rates for DBU from Benlate Incubations at Various Temperatures and Humidities[a]

formulation	23 °C			37 °C		
	32%	75%	96%	32%	75%	96% (28)[b]
DFE	0.0000	0.0001	0.0022*[c]	0.0001	0.0012*	0.0026*
DFF	0.0000	0.0001	0.0022*	0.0001*	0.0014*	0.0030*
DFG	0.0001	0.0002*	0.0023*	0.0001*	0.0013*	0.0040*
DFZ	0.0000	0.0002*	0.0025*	0.0001*	0.0012*	0.0037*
WPA	0.0000	0.0000	0.0032*	0.0000	0.0006*	0.0061*
WPD	0.0000	0.0001	0.0030*	0.0000	0.0008*	0.0065*
av WP	0.0000	0.0000	0.0025*	0.0000	0.0007*	0.0063*
av DF	0.0000	0.0001*	0.0028*	0.0001*	0.0013*	0.0032*

[a] Formation rate constants (mol of DBU/mol of benomyl/day) for each formulation at each temperature and humidity level. In cases where the rate over the 56 day incubation decreased after time, the maximum rate is given. The last time point used for the straight line regression is indicated in parentheses. [b] Analyzed after only 28 days of storage. [c] *, significant increase ($p < 0.05$), compared to capped control samples. See text for percentage DBU values.

Benomyl and MBC Concentrations. Benomyl concentrations in unincubated samples varied widely, ranging from 0.9% (WPE) to 50.9% (DFH9), which well inversely correlated with the DBU and MBC concentrations, as might be expected. However, total benomyl concentrations (benomyl plus MBC) did not correlate well with DBU concentrations; total benomyl concentrations ranged from 46.8 to 55.3%, with an average of 51.9% (Table 2). Postincubation benomyl recovery did not vary with DBU content either, but was consistent with formulation type. As expected, the ratio of benomyl to MBC decreased after incubation, falling to 0.025 in the iDFUS2 sample, to 1.32 in the iDFG sample, and

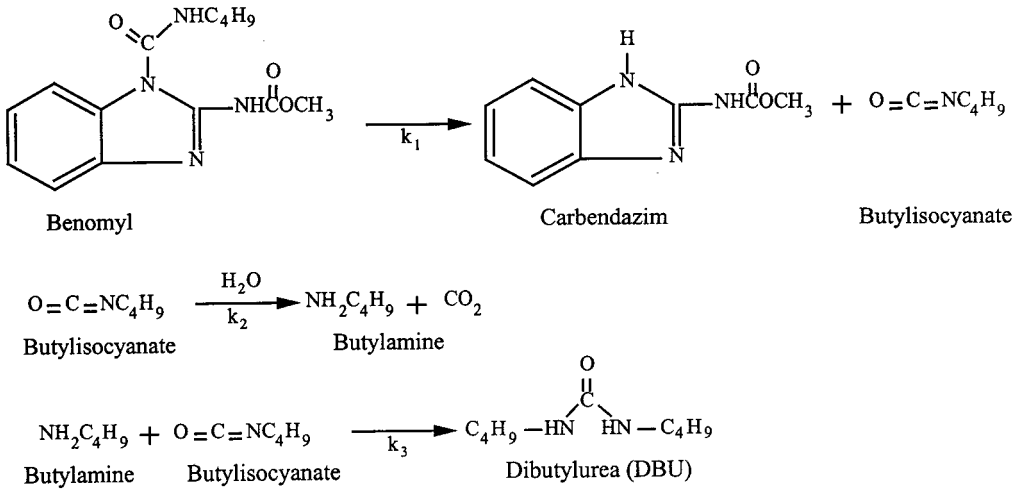

Figure 1. Reaction pathway for the formation of DBU from benomyl and water.

to 0.837 in the iWPA sample, implying degradation of benomyl and release of BIC for subsequent reactions.

DBU Formation Kinetics. The data shown in Table 3 summarize the kinetics of DBU formation in the formulation samples stored at the various temperatures and humidities for 56 days except for the 37 °C samples, which were carried out to only 28 days. Formation rate constants were generally larger for the WP samples than for the DF samples, with the largest observed for the WP sample at 37 °C and 96% RH. Compared to capped controls, significant differences were observed for all 37 °C samples exposed to 75 and 96% RH; fewer differences were observed for the 23 °C samples. As might be expected, rate constants generally increased with increasing temperatures and humidities. More DF samples than WP samples showed significant differences, however, which occurred at generally lower temperatures and humidities than the WP samples. The WP formulations, therefore, required a high-humidity environment to rapidly form DBU, whereas the DF formulations did not. Although temperature caused a significant increase in the initial rate of DBU formation in the WP formulations, humidity was clearly the dominant factor associated with DBU formation.

DISCUSSION

The overall reaction scheme for DBU formation from benomyl is shown in Figure 1. The rate of the initial reaction (k_1) for the conversion of benomyl to MBC has been shown to be small in water through the use of radiolabeled benomyl (Baude et al., 1974). This may be explained by the low solubility of benomyl in aqueous solutions (3.8 ppm at 20 °C). When prepared as a 250 ppm solution (drench rate), only 1.5% of the benomyl will be dissolved at any time. Given that the half-life of benomyl in water is ~7 h, it would take an extended period of time for the benomyl to completely decompose (Calmon and Sayag, 1976). Although the decomposition rate in water is slow, the rate in organic solvents is very rapid. The consequences of the hydrophobic environments created by the various surfactants found in the formulations on the degradation rate of benomyl in water are unknown. Additionally, the surface chemistry system investigated herein may act quite differently from the micellar system predicted by solution chemistry. Differences in DBU formation rates between the granular Benlate DF50 formulation and the smaller particles in the Benlate WP50 formulation may be a direct consequence of Benlate particle size.

Butylisocyanate has been shown to react with water to give dibutylurea in high yield (50% of theoretical). The half-life of the BIC/water reaction products as determined by GC/NP (825 s) was similar to other reported values conducted by NMR analysis (945 s; Moye et al., 1994). These results indicate the high reactivity of butylisocyanate with water to form the dialkyurea (Figure 1; k_2, k_3).

Although GC/NP analysis revealed only a single product of the BIC reaction with water, NMR analysis showed the presence of a second compound, the salt of butylamine (Moye et al., 1994). The NMR analysis failed to detect butylamine in the reaction mix after 1.5 min. This indicates that the reaction rate for the decarboxylation of butylcarbamic acid to butylamine and the subsequent reaction of butylamine with a second molecule of BIC are extremely fast (k_3). Kinetic experiments on BIC added to a solution of butylamine in D_2O revealed only trace amounts of each after 1.5 min (Moye et al., 1994). Thus, butylamine appears to be rapidly scavenged by available BIC. The overall rate-limiting step in the formation of DBU from benomyl appears to be the initial elimination of BIC from benomyl to liberate MBC (k_1).

The stoichiometric maximum yield of DBU from a 50% benomyl product is 14.8 wt %. The recovered DBU in the 96% RH and 37 °C incubates demonstrates the high efficiency in these reactions. The highest DBU levels found on analysis tend to occur in WP lots. Perhaps the larger question raised by this observation is the ultimate product of the released BIC from the DF lots. It seems likely that the degradation to MBC proceeds to completion in both the 37 °C and 96% RH DF and WP incubates. If so, then BIC liberated from the DF that does not form DBU is unaccounted for in the experiment. The higher levels of *n*-butylacetamide and *N*-butylformamide in the DF formulations as compared to the WP formulations may partially explain the missing liberated butylcarbamoyl moiety (Moye et al., 1994).

The higher levels of moisture available in the Benlate DF formulations may have a significant role in the DBU formation rates at the low ambient moisture conditions.

The moisture content of the powdered redried starch A, which comprises 25 wt % of the Benlate DF50, is similar to that of the formulated product. Water tends to be held or bound with solid matrixes by both chemical and physical forces and in the Benlate DF50 formulations probably exists as free water, loosely held by absorptive forces acting on the surface of the formulation particles, or within a colloidal gel formed by starch where the tortuous diffusion path of intriculated water hinders removal (Potter, 1978). Still more difficult to remove is water that is chemically bound in the form of hydrates of sugar monomers. The starch then, may act as a reservoir for water to initiate DBU formation in Benlate DF50 formulations even when stored at low relative humidities.

The high levels of moisture weight gain upon incubation were surprising because none of the samples appeared to have visible moisture either on the vial or in the powder. The higher moisture levels found in the WP formulation may be related to the increased surface area offered by the formulation and may be partially responsible for the higher DBU levels found after high-humidity incubations.

The total benomyl found by HPLC analysis of Benlate formulations was consistent with the labeled 55% active ingredient content specified for the DF50 formulation and 53% active ingredient content specified in the WP50 product. The somewhat lower average benomyl recoveries from the incubated samples may have resulted from moisture weight gain during the incubation. When the calculated weight gain based on hygroscopic measurements is used to adjust the benomyl recovery, the WP50 recovery becomes 47.8% and the DF recovery becomes 55.4%. This does not consider other factors that may affect the final product weight, such as loss of CO_2 during benomyl degradation or volatilization of BIC or other formulation constituents.

The rate of DBU formation was found to depend significantly on the humidity ($p < 0.001$) and the temperature ($p < 0.001$) and also varied by formulation type. Significant temperature, humidity, and formulation type interactions resulted from variations among the formulation types response to the various levels of each factor. The DF formulations tended to have higher rates at the 32 and 75% RH levels, whereas the WP formulations tended to have higher rates at 96% RH. Higher temperature potentiates the 32 and 75% RH more than the 96% RH for the DF ($p < 0.01$). This is consistent with the findings of Tang and Song (1996). The DF formulations are affected by temperature at the 32 and 75% RH more so than the WP formulations.

These findings may yield considerable insight on the appearance of DBU in Benlate formulations. DBU has been shown to form by decomposition of benomyl during storage of the product and is coupled to the disappearance of intact benomyl and concomitant formation of MBC. Elevated temperature and humidity conditions have been shown to promote the formation of DBU in both WP and DF formulations. During the production, transportation, storage, and use of Benlate products, several opportunities exist for the temperature and humidities necessary for the formation of DBU. The manufacture of the granular DF formulation requires the addition of water and subsequent air-milling at elevated temperatures, conditions highly favorable for DBU formation. Recycling of out-of-specification product could further increase the DBU levels of the final product. Furthermore, because none of the six tested sealed Benlate DF packages were found to be completely airtight, with some packages having grossly unsealed inner bags, the possibility of moisture penetration during transportation or storage exists. Transportation of product in truck or rail car, as well as storage in farm chemical sheds, provides a potential high-temperature environment conducive to DBU formation. Finally, the product is prepared and applied in a relatively high concentration aqueous suspension, where the possibility exists for further DBU formation before application. It should be noted here, however, that in the studies described in this paper, the Benlate formulation was exposed to a constant humidity as it was spread out in vials as a thin layer of material. Formulation in bags being stored or transported would not be exposed to such a controlled humidity and would be expected to behave somewhat differently.

The role of DBU as the causative agent in the reported Benlate-related phytotoxicity was not specifically addressed by this research. The primary goal of this research was to determine the chemical changes that occur to Benlate during simulated storage conditions. While DBU and DBU-containing Benlate have been shown to affect several plant species when applied at drench rates comparable to those of analyzed formulation samples, the lower foliar application rates appear to have no effect. However, given the levels of DBU found in formulations and its demonstrated action on the plants studied, the continued investigation of DBU is essential to fully understanding Benlate-related phytotoxicity.

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