JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Chlorine and Chlorine Dioxide Treatment to Reduce or Remove EBDCs and ETU Residues in a Solution

E. HWANG,[†,‡,||] JERRY N. CASH,*[,†,‡,§] AND MATTHEW J. ZABIK[‡,§]

Department of Food Science and Human Nutrition, Institute for Environmental Toxicology, and National Food Safety and Toxicology Center, Michigan State University, East Lansing, Michigan 48824

Calcium hypochlorite ($Ca(OCl)_2$) and chlorine dioxide (ClO_2), common disinfecting and bleaching chemicals used in the food industry, are potent oxidizing agents. In this paper, the degradation effects of chlorine dioxide on mancozeb and ethylenethiourea (ETU) residues were investigated in a model system and compared with those of liquid chlorine, under various conditions such as differing concentration, pH, reaction time, and temperature. All samples were analyzed for residues by GLC and HPLC. Rate of mancozeb degradation was dependent on pH, with pH 4.6 being the most effective. Mancozeb residues decreased 40−100% with chlorine and chlorine dioxide treatments. ETU residue concentrations in mancozeb solutions were monitored over 60 min. Under controlled conditions, the ETU residue concentrations increased up to 15 min reaction time and then decreased in all three pH ranges. Treatment with both chlorine and chlorine dioxide at pH 4.6, yielded no ETU residues at both 10 and 21 °C. The results show that chlorine dioxide gives excellent degradation effects at lower concentrations than liquid chlorine.

KEYWORDS: Pesticide; degradation; mancozeb; ethylenethiourea; calcium hypochlorite; chlorine dioxide

INTRODUCTION

Ethylene bisdithiocarbamates (EBDCs) are important organic fungicides that are used in a variety of crops (*1*). Approximately one-third of all fruits and vegetables in the United States are treated with EBDCs (*2*). Mancozeb is a polymeric complex of ethylenebisdithiocarbamate manganese and zinc salt. It is one of the most widely used EBDCs for control of various fungal pathogens in fruits and vegetables. EBDCs have been regarded as relatively harmless because of their low acute toxicity to mammals, but there is some concern about ethylenethiourea (ETU), a degradation product of EBDCs. It can be formed in EBCDs during manufacture or storage or in food containing EBDC residues during cooking and processing at elevated temperature and high humidity. ETU is a relatively stable and very polar metabolite (*3*). Because of the report of its carcinogenic, mutagenic, and goitrogenic effects in laboratory animals, ETU has become a major human health concern among consumer groups.

Chlorination has been used for many years by the food industry as a sanitizing and disinfecting agent (*4*). Chlorine as sodium, potassium, or calcium hypochlorite has been used for many years by the food industry and public water suppliers as their principal sanitizing and disinfecting agent (*5*). Hypochlorites are powerful disinfectants which are active against a wide spectrum of organisms, and they are nontoxic to humans at low concentrations (*6*). Many organic compounds present in foods and water treated with chlorine are subjected to chlorination reactions. When chlorine is applied onto organic molecules, their hydrophobicity or lipophilic nature increases. This in turn often increases the toxicity and bioaccumulation of these compounds. There are potential health hazards connected with the use of chlorine because reaction products that are formed have toxic activity such as mutagenicity, teratogenicity, or carcinogenicity (*7*).

Chlorine dioxide is a gas that is soluble in water. This offers many advantage over chlorine as a biocide in water systems. Chlorine reacts with organic materials to form chloroform and trihalomethane. In contrast, chlorine dioxide is not a chlorinating reagent so no chloroform or other trihalomethanes are formed (*8*). Chlorine dioxide has been used in the public water supply and food industries. Several reports have addressed the use of chlorine dioxide as a bacteriocide to reduce bacterial populations both in poultry chiller water and on poultry carcasses. Chlorine

* To whom correspondence should be addressed at 334 G. M. Trout Food Science and Human Nutrition Building, East Lansing, MI, 48824. Tel: (517) 353-5339. Fax: (517) 353-1641. E-mail: jcash@msu.edu.
† Department of Food Science and Human Nutrition.
‡ Institute for Environmental Toxicology.
§ National Food Safety and Toxicology Center.
|| Current address: Department of Food Science and Human Nutrition, University of Illinois at Urbana-Champaign, Urbana, IL. 61801.

10.1021/jf020307c CCC: $22.00
Published on Web 07/03/2002

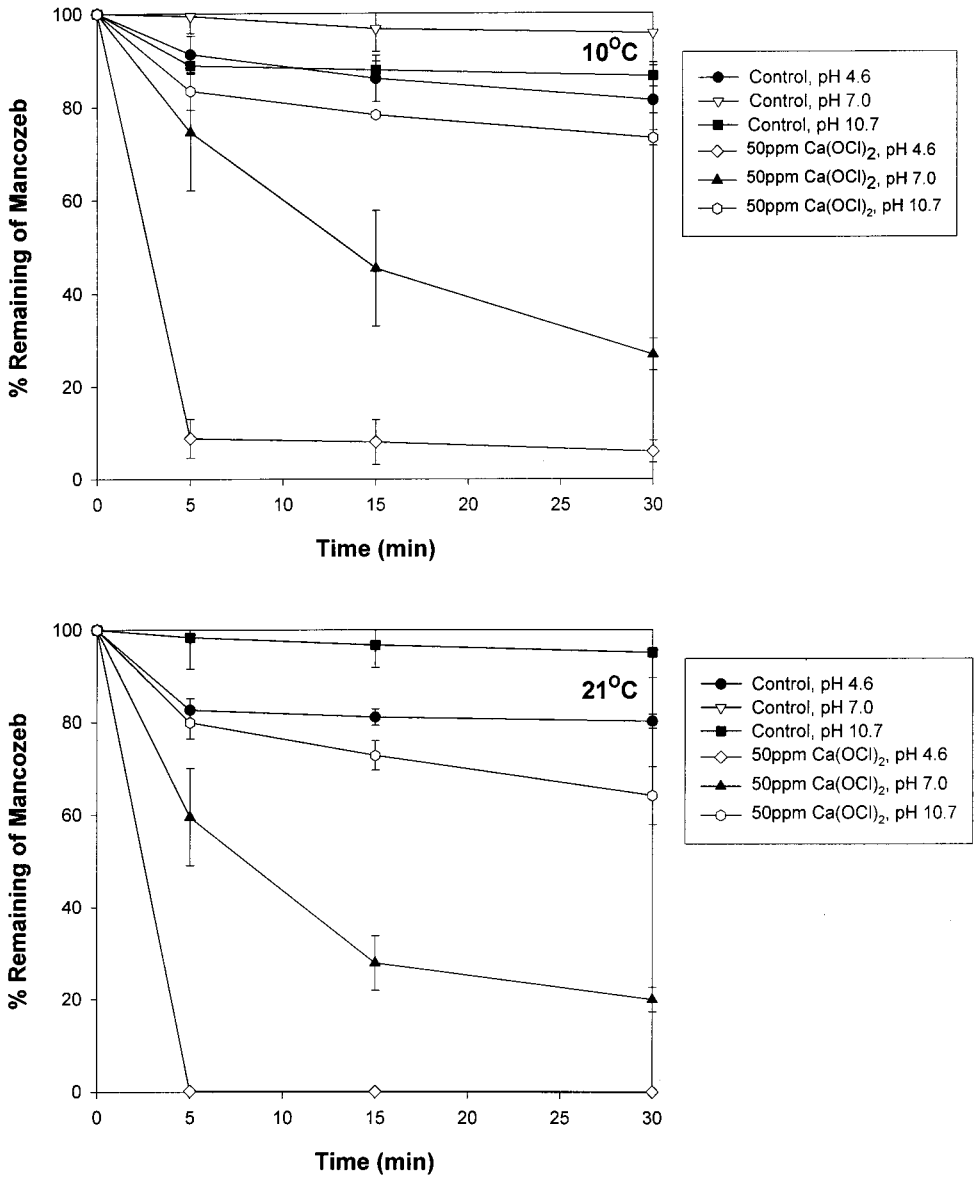

Figure 1. Effect of 50 ppm $Ca(OCl)_2$ on the degradation of 2 ppm mancozeb at 10 and 21 °C.

dioxide has proved to be an excellent biocide and an effective oxidant in drinking water, cooling water, wastewater, and odor-control applications. Chlorine dioxide achieved faster kill of microorganisms at lower concentrations than did other chorine-based sanitizers (*9*). It has been reported that pesticides can be removed by chlorine dioxide, particularly aldrin and methoxychlor. Herbicides such as paraquat and diquat are eliminated within a few minutes at a pH higher than 8 (*10*). The U.S. Environmental Protection Agency (EPA) considers chlorine dioxide as the first choice of disinfectant to replace liquid chlorine (*11*).

The objective of this study was to determine the effectiveness of different chemical oxidants on degradation of mancozeb and ETU in aqueous solution using calcium hypochlorite and chlorine dioxide treatment.

MATERIALS AND METHODS

Materials. Mancozeb standard was obtained from Rohm & Haas (Philadelphia, PA). ETU standard was obtained from Aldrich Chemical Co. (Milwaukee, WI). The stock solutions of mancozeb and ETU were prepared in distilled water at concentrations of 1 μg/mL. The standards were protected from light and stored in a refrigerator at 4 °C. Chlorine solutions were prepared from calcium hypochlorite (Aldrich). Sodium thiosulfate, sodium sulfate, potassium iodide, potassium indigo trisulfonate, potassium fluoride, and ammonium chloride were all reagent grade. All organic solvents used for preparation of stock solution and HPLC were distilled-in-glass grade.

Methods. Laboratory studies were conducted in a model system to determine the effect of (1) calcium hypochlorite at three concentrations (50, 250, and 500 ppm) and chlorine dioxide at two concentrations (5 and 10 ppm); (2) treatment at three pH levels (4.6, 7.0, and 10.7); and (3) treatment at two temperatures (10 °C and ambient temperature (21 °C)).

Aqueous solutions at pH 4.6 (0.2 M sodium acetate), pH 7.0 (0.2 M sodium phosphate), and pH 10.7 (0.2 M carbonate/bicarbonate) were prepared. Degradation of mancozeb was studied over a 30-min period because the typical water contact time for apples in a commercial plant is about 10−15 min and under normal conditions would rarely exceed 30 min. There were three replications per treatment.

Aqueous Solution Study. For a chlorine source, calcium hypochlorite stock solution (5000 ppm) was added to each pH solution to bring

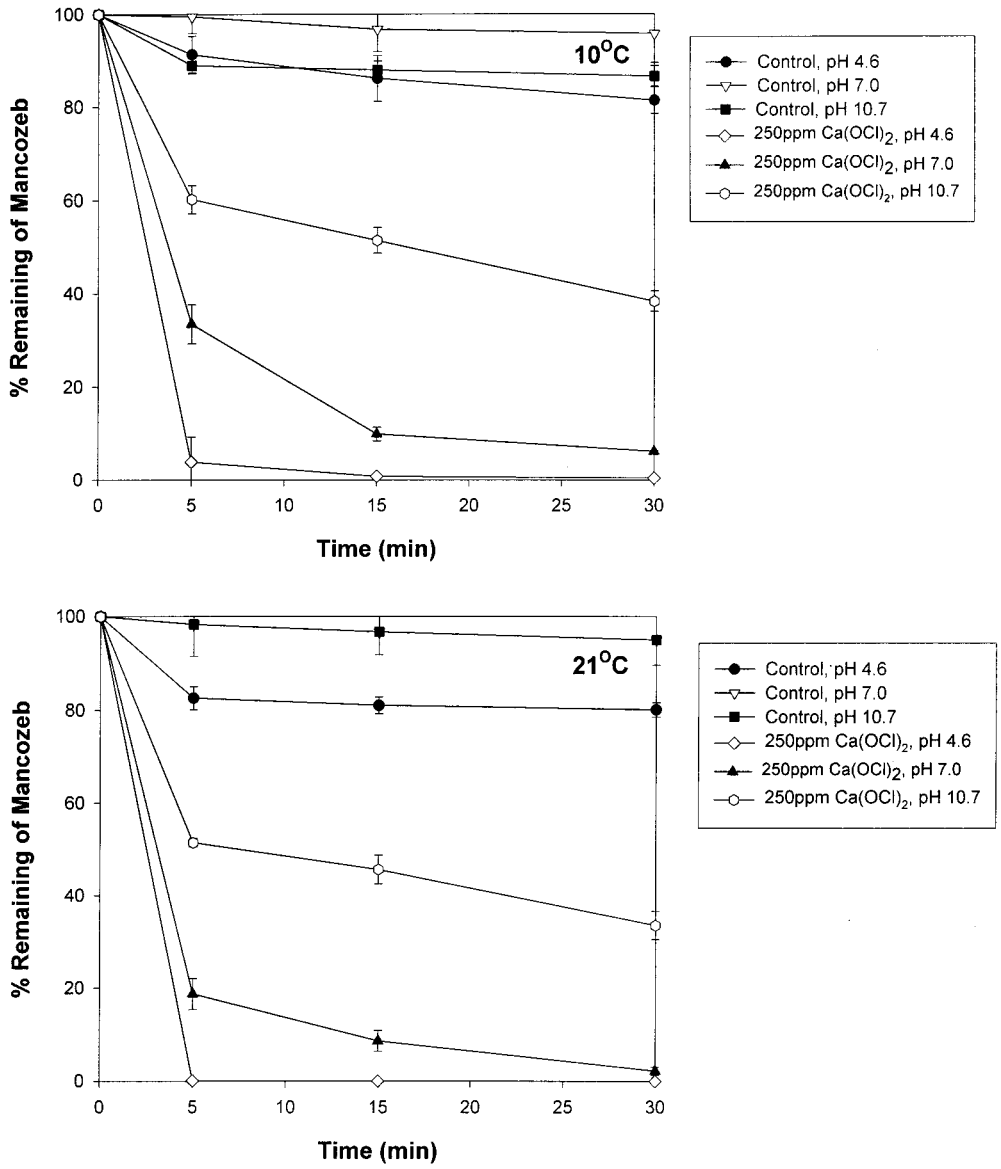

Figure 2. Effect of 250 ppm $Ca(OCl)_2$ on the degradation of 2 ppm mancozeb at 10 and 21 °C.

the final chlorine concentration to 50, 250, or 500 ppm. Each pH solution was spiked with the mancozeb stock solution to give a final concentration of 2 ppm. Total available chlorine was determined using the iodometric method (*12*).

Chlorine dioxide was generated in the laboratory using the manufacturer's (S. C. Johnson Professional, Racine, WI) instructions as follows. A 100-mL aliquot of the stock 2% Oxine FP solution was added to a 200-mL French square screw-capped bottle. Food grade phosphoric acid (25 mL, 75% w/w) was added, the bottle was sealed, and the mixture was allowed to generate chlorine dioxide for 5 min with a magnetic stirrer to ensure thorough mixing. After 5 min, the concentrated chlorine dioxide was transferred into 15 L of each pH solution in a closed container to serve as a stock solution. For 5 or 10 ppm of chlorine dioxide, 2 or 4 L of stock solution, respectively, were diluted to 30 L with each pH buffer solution. The final concentration of chlorine dioxide was determined using the HACH chlorine colorimeter before and after each sampling run.

Mancozeb and ETU Residue Analyses. Mancozeb residues were analyzed as carbon disulfide (CS_2) by gas−liquid chromatographic headspace analysis (*13*). Sample aliquots of 20 mL were transferred at 0, 5, 15, and 30-min intervals into sample bottles. A 0.1 M sodium thiosulfate solution was immediately added to the samples to quench the reaction. Stannous chloride (40 mL, 1.5%) in 5 M HCl was added, and the bottles were immediately sealed with a crimped-septum. A 50-mL aliquot of a 1 mg/mL thiophene solution was injected as an internal standard into each bottle, and the bottles were incubated at 70−80 °C in a water bath for 15 min. Bottles were removed and agitated by hand for 2 min. Bottles were replaced in the water bath with repeated shaking for 1 h. A 100-mL sample was removed with a gastight syringe from the headspace of each bottle and injected into the GC.

ETU residues were determined by using a modification of the HPLC method published by Ahmad et al. (*13*). A 20-mL portion of sample was weighed into an Erlenmeyer flask, then 8 g of potassium fluoride and 0.6 g of ammonium chloride were added. This mixture was extracted 2 times with 50 mL of dichloromethane. The dichloromethane layer was passed through a bed of 25 g of anhydrous sodium sulfate, collected in a round-bottom flask, and evaporated to dryness on an automated Zymark Turbovap evaporator at 40 °C. The residue was dissolved in 3 mL of distilled water and 50 μL was injected into an HPLC column between standards.

Chromatographic Analyses. Mancozeb residues were detected and quantified using a Hewlett-Packard Series II 5890 gas chromatograph (GC) equipped with a flame photometric detector (FPD) in the sulfur

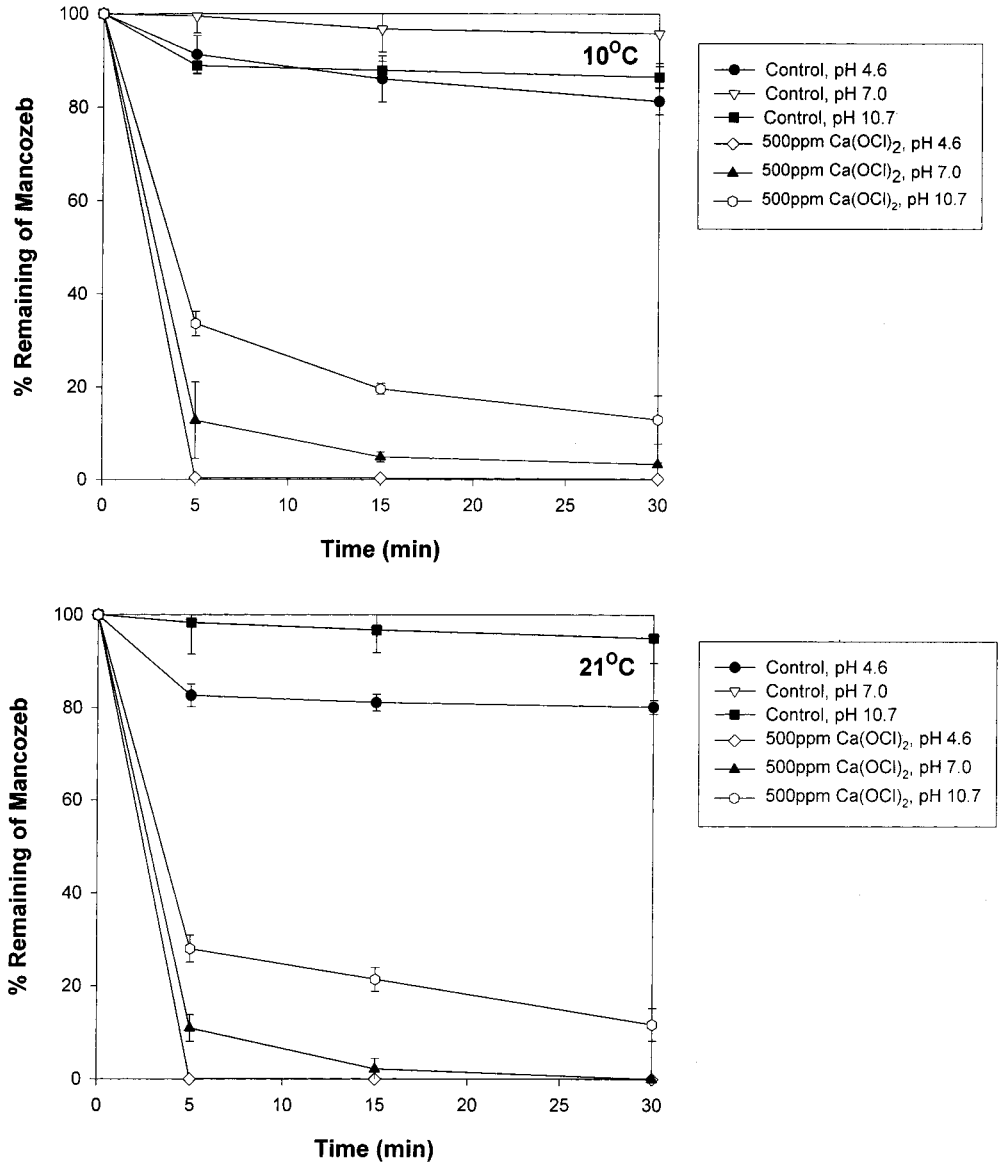

Figure 3. Effect of 500 ppm $Ca(OCl)_2$ on the degradation of 2 ppm mancozeb at 10 and 21 °C.

mode. The GC was equipped with a Supel-Q-Plot fused silica capillary column (30 m long × 0.53 mm i.d.) with a film thickness of 0.25 μm (Supelco Inc., Bellefonte, PA). The oven temperature was programmed isothermally at 80 °C, while the injector and detector temperatures were 230 °C and 300 °C, respectively. Helium and nitrogen were used as the GC carrier gas and makeup gas, respectively. Carrier gas flow through the column was 20 mL/min. Integration was carried out with HP Chemstation software interfaced to the GC.

ETU residues were detected and quantified using a liquid chromatograph with a Hypersil BDS C_{18} column (250 mm × 4.6 mm, 5-μm particles), a Hypersil BDS C_{18} guard column (10 mm × 4.6 m, 5-μm particles), and a UV detector set at 240 nm. The mobile phase was 0.72% butylamine in distilled water at pH 3.0−3.2. A M-45 Waters HPLC pump (Waters Associates, Inc., Milford, MA) was used for solvent delivery at a flow rate of 0.5 mL/min. After the system was stabilized, 50-μL samples were injected.

Statistical Analysis. All determinations were replicated three times. Means, standard deviations, mean square errors, two factor ANOVA, and correlation and interaction of main effects were calculated using Sigma Stat computer software 1.0 (Jandel Corp., San Rafael, CA). Appropriate comparisons were made using the Student−Newman−Keuls method for multiple comparisons. A $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

In the GLC analysis, carbon disulfide appeared as a single sharp peak at a retention time of 5.1 min. Mancozeb was stable at pH 7.0 at both 10 and 21 °C with very little degradation due to hydolysis. Between 95 and 99% (10 °C) and 95 and 97% (21 °C) residual mancozeb remained after 30 min. Mancozeb was relatively less stable at pH 4.6 and 10.7, with about 78 and 80% remaining, respectively after 30 min at ambient temperature (**Figure 1**). This indicates mancozeb is less stable under basic and acidic conditions than at neutral condition.

Degradation of Mancozeb by Calcium Hypochlorite. Degradation of mancozeb by calcium hypochlorite solution was greatest at pH 4.6 and decreased with increasing pH. The chlorine treatment at pH 10.7 was the least effective at both 10 and 21 °C. Its degradation was only about 27 and 40% after 5 min at 50 ppm calcium hypochlorite (**Figure 1**). In 50 ppm calcium hypochlorite solution, mancozeb was completely degraded at pH 4.6 after 5 min (**Figure 1**). The 50 ppm chlorine treatment at pH 10.7 was the least effective, with

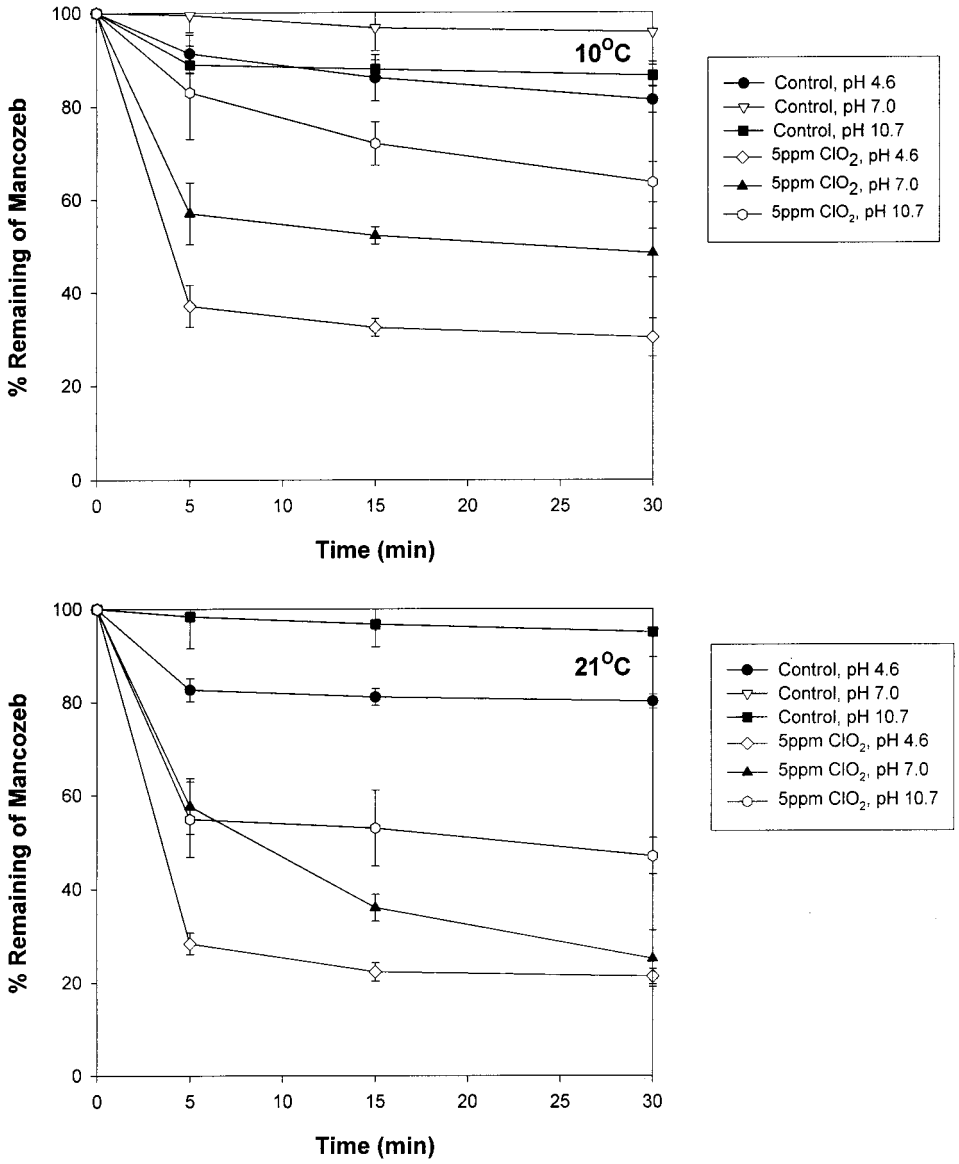

Figure 4. Effect of 5 ppm ClO_2 on the degradation of 2 ppm mancozeb at 10 and 21 °C.

degradation only about 20 and 36% after 5 and 30 min, respectively. Lower temperature decreased the degradation of mancozeb at all pH ranges during the entire sampling period (**Figures 1**−**3**).

Treatment with calcium hypochlorite at 250 and 500 ppm significantly ($p < 0.05$) increased the rate of degradation of mancozeb in all three pH treatments and at both temperatures. No mancozeb remained after both 250 and 500 ppm calcium hypochlorite treatment at pH 4.6 and ambient temperature after 5 min (**Figures 2** and **3**). At pH 10.7, almost 50 and 30% of mancozeb residues remained after 5 min at 250 and 500 ppm calcium hypochlorite, respectively, at ambient temperature. Again, the most effective treatment was calcium hypochlorite at 500 ppm in the pH 4.6 solution, and pH 10.7 was the least effective treatment.

Degradation of Mancozeb by Chlorine Dioxide. Chlorine dioxide is a polar gas that readily dissolves in, but does not react with, water (*14*). This compound has a larger oxidation capacity than that of calcium hypochlorite. Degradation of mancozeb by chlorine dioxide showed a pattern similar to that by calcium hypochlorite treatment, however, chorine dioxide was more effective than calcium hypochlorite treatment (**Figures 4** and **5**). As can be seen in **Figures 6** and **7**, when chlorine dioxide and liquid chlorine were used to degrade mancozeb residues, the required amount of chlorine dioxide was lower than that of liquid chlorine. Maximum degradation of mancozeb by chlorine dioxide was observed at pH 4.6. In 5-ppm chlorine dioxide treatment, between 62 and 78% of mancozeb remained after 5 min at both 10 and 21 °C. Chlorine dioxide at 10 ppm significantly ($p < 0.05$) increased the rate of degradation of mancozeb in pH 4.6 at both temperatures. However, there was no significant ($p < 0.05$) difference in the degradation of mancozeb between 5 and 10 ppm chlorine dioxide at pH 7.0 and 10.7 at both temperatures.

Chlorine in water is hydrolyzed very easily to form hydrogen chloride (HCl) and hypochlorous acid (HOCl).

$$Cl_2 + H_2O = HOCl + H^+ + Cl^-$$

$$HOCl \leftrightarrow H^+ + OCl^- \ (pK_a = 7.49)$$

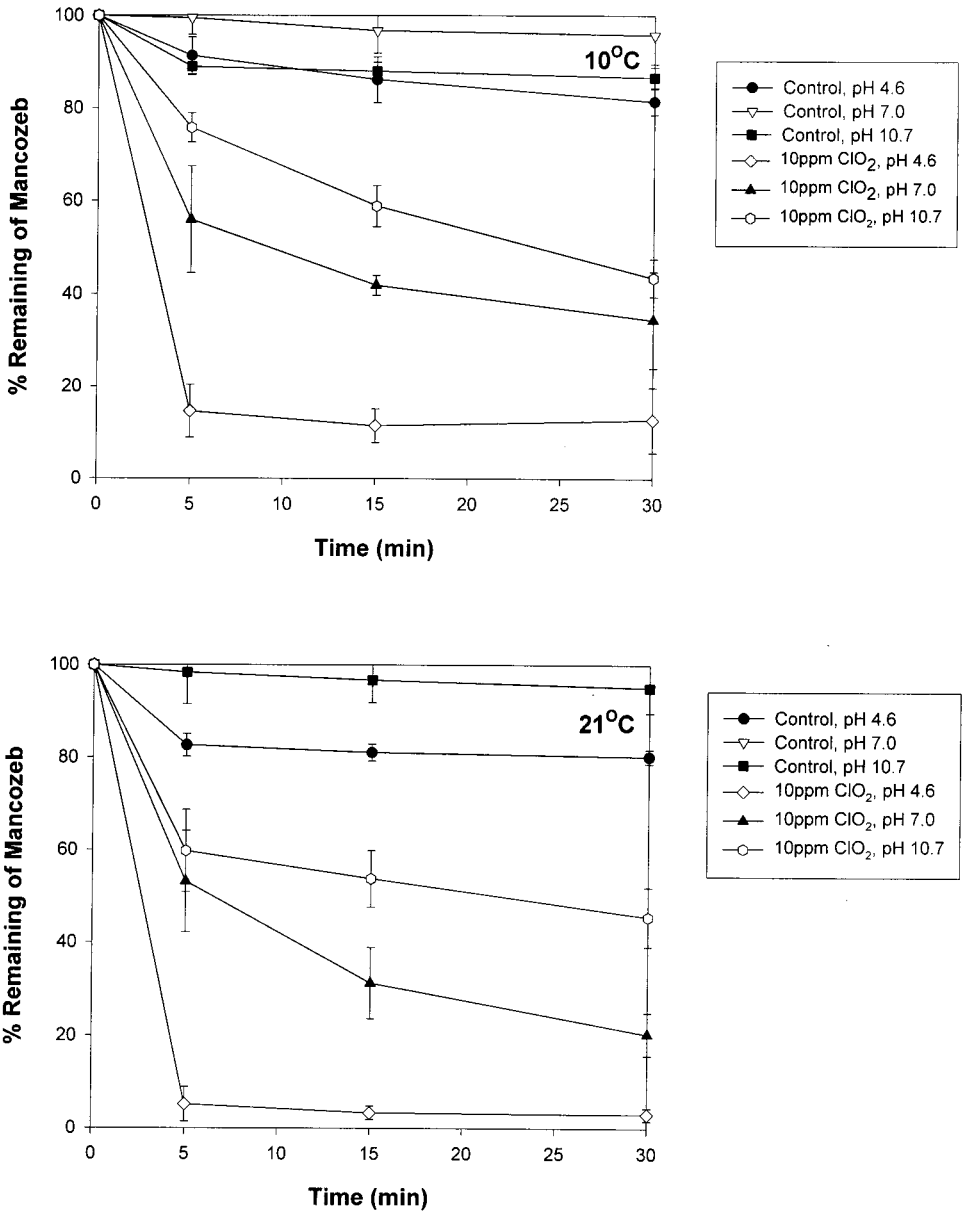

Figure 5. Effect of 10 ppm ClO_2 on the degradation of 2 ppm mancozeb at 10 and 21 °C.

In water treatment HOCl and hypochlorite (OCl^-) coexist and their relative concentrations are pH dependent. At acidic pH, HOCl is the predominant species, representing greater than 90% of the chlorine in solution at 25 °C. However, at alkaline pH, OCl^- is the major species (*15*). Because the pK_a of HOCl and its anion is 7.49, this is only true at basic pH values greater than 7.49. HOCl plays a main role in bactericidal and disinfecting function. The efficiency of HOCl is nearly 80 times as high as that of OCl^-. The higher the pH is, the lower the ratio of HOCl and the weaker the activity is, and the poorer the disinfection effects are (*16*).

The mechanism of chlorination and oxidation of organic compounds by chlorine dioxide are not known. There are two inaccuracies here. First of all, as noted above, chlorine dioxide is not considered to be a chlorinating agent. When chlorination (introduction of chlorine into an organic compound) does occur in the presence of chlorine dioxide it is usually because diatomic chlorine is present as a contaminant. Second, the general

Table 1. ETU Conversion from Samples Fortified with Mancozeb (After 15 Min Reaction Time)

treatmen	pH	EBDC added (ppm)	ETU found[a] (ppb)	% conversion[b]
control	4.6	2.0	14.77 ± 0.91	0.74
	7.0	2.0	21.93 ± 1.11	1.10
	10.7	2.0	19.07 ± 0.75	0.95
50 ppm $Ca(OCl)_2$	4.6	2.0	N. D.[c]	
	7.0	2.0	7.60 ± 0.89	0.38
	10.7	2.0	9.53 ± 0.67	0.48
250 ppm $Ca(OCl)_2$	4.6	2.0	N. D.	
	7.0	2.0	N. D.	
	10.7	2.0	5.20 ± 0.44	0.26
500 ppm $Ca(OCl)_2$	4.6	2.0	N. D.	
	7.0	2.0	N. D.	
	10.7	2.0	8.10 ± 0.72	0.41
5 ppm ClO_2	4.6	2.0	N. D.	
	7.0	2.0	7.60 ± 0.89	0.38
	10.7	2.0	9.53 ± 0.67	0.48
10 ppm ClO_2	4.6	2.0	N. D.	
	7.0	2.0	N. D.	
	10.7	2.0	5.20 ± 0.44	0.26

[a] Means with the same superscript are not significantly different ($p > 0.05$). Means with standard deviations; $n = 3$ for all treatment. [b] % ETU conversion was calculated by (wt. ETU/wt. EBDC) × 100. [c] N. D. = None detected. This represents a value <5ng/g which is the method of detection limit for ETU in solutions.

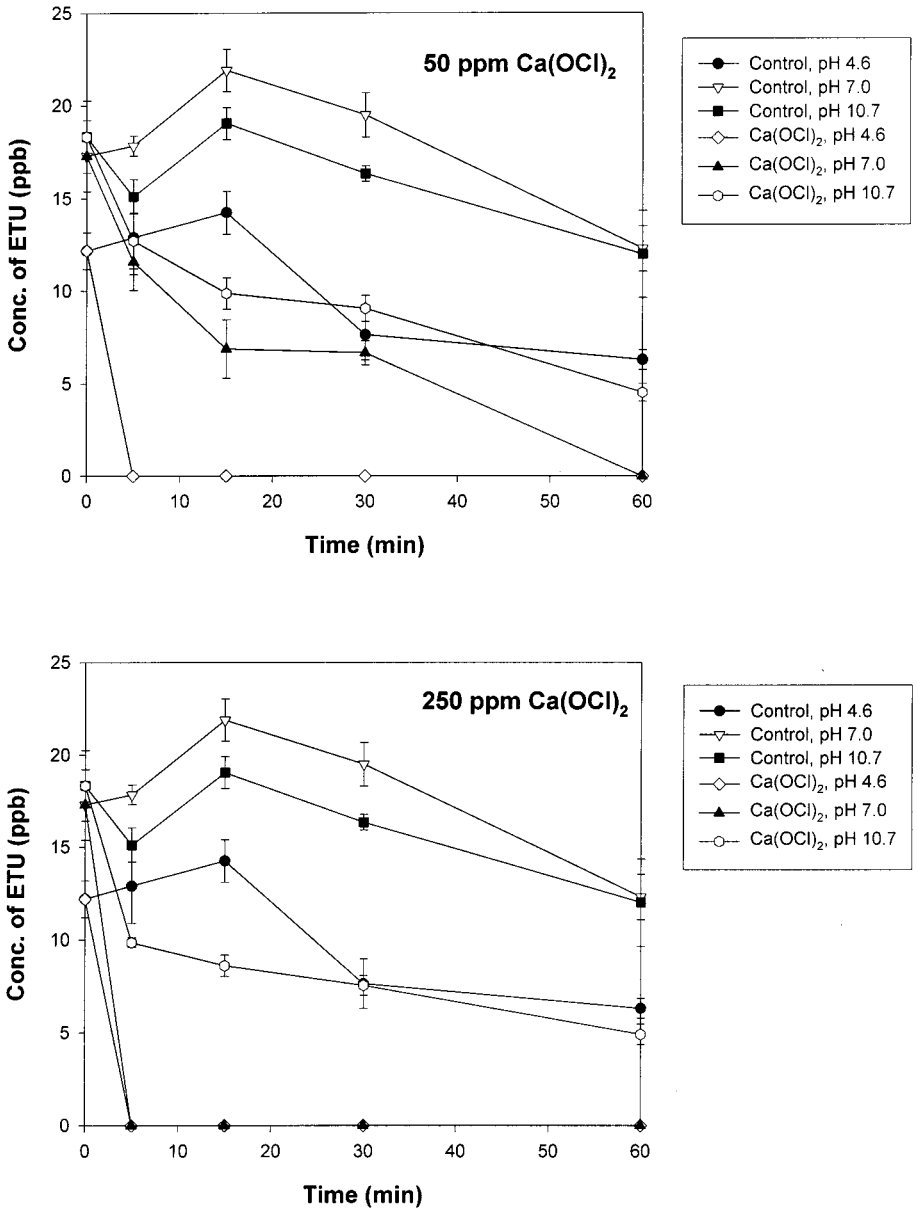

Figure 6. Effect of $Ca(OCl)_2$ on the conversion of 2 ppm mancozeb to ETU at 21 °C.

mechanism of oxidation of organic compounds by chlorine dioxide is known. Chlorine dioxide is a neutral radical species. As such it is a good oxidant and ultimately undergoes a five-electron reduction to form chloride ion and water. Chlorination in aqueous solutions may occur indirectly through a progressive reduction of chlorine dioxide, which passes through the HOCl stage (*15*).

Conversion of Mancozeb into ETU. In the HPLC analysis, ETU appeared as a peak with a retention time of 10.4 min. The degradation of mancozeb to ETU in solution due to hydrolysis, chlorine, and chlorine dioxide treatment is shown in **Table 1** and **Figures 6** and **7**. It was found that the rate of decomposition of mancozeb to ETU was influenced by pH. The total yield of ETU was decreased when the pH was lowered from 7.0 or 10.7 to 4.6. At pH 7.0, the initial ETU concentration was 17.3 ppb, which increased to 21.9 ppb after 15 min and then decreased to 12.3 ppb after 60 min. In the case of pH 4.6, the initial ETU concentration was 11.9 ppb, which increased to 14.3 ppb after 15 min and then decreased to 5.3 ppb after 60 min. This shows that acidic pH is much more effective in reducing the conversion rate of mancozeb into ETU compared with neutral or alkaline pH ranges. In processing, acidic treatment can be a preventive method in ETU production. Engst and Schnaak (*17*) reported that ethylenebisdithiocarbamic acid readily forms ETU under highly alkaline conditions (pH 10.5). As shown in **Figures 6** and **7**, conversion of mancozeb to ETU reached a maximum at 15 min reaction time and then decreased for all three pH ranges.

The rate of degradation of the EBDC to ETU was influenced by temperature, reaction time, and pH of the system.

CONCLUSIONS

The objective of this study was to determine the effectiveness of chlorine and chlorine dioxide treatment on the dissipation of mancozeb in buffered solution. It is considered that calcium

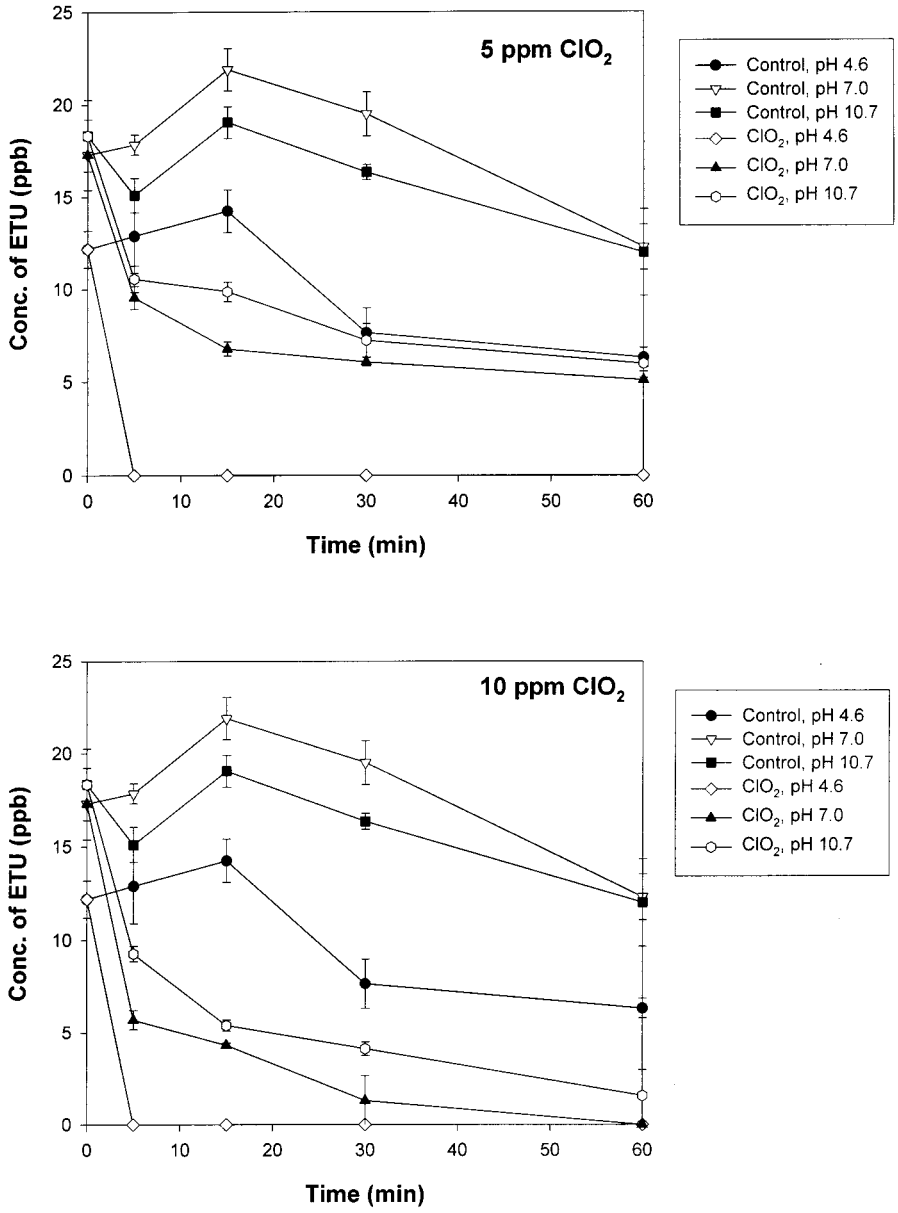

Figure 7. Effect of ClO_2 on the conversion of 2 ppm mancozeb to ETU at 21 °C.

hypochlorite and chlorine dioxide treatments are effective to reduce or remove ETU residues as well as EBDCs. Mancozeb residues in model system solutions decreased 40−100% with chlorine treatment. The rate of degradation of mancozeb increased at acidic pH and ambient temperature. Degradation of ETU by calcium hypochlorite and chlorine dioxide was greatest at pH 4.6 and lowest at pH 10.7. This showed patterns similar to the degradation of mancozeb by these oxidants. With chlorination at pH 4.6, no ETU residues were detected at both calcium hypochlorite and chlorine dioxide.

This study indicate that chlorine dioxide was better than liquid chlorine in the degradation of mancozeb residue.

A model system was developed which was shown to be effective in monitoring the degradation or disappearance of mancozeb through the use of various pH, temperature, chlorine, and chlorine dioxide treatments. These treatments showed potential for the removal of pesticide residues on fruit and in processed products.

LITERATURE CITED

(1) U.S. Environmental Protection Agency (EPA). *Preliminary Risk Assessment.* Environmental Science Division Reg. 5-85173535598; U.S. Government Printing Office: Washington, DC, 1989; pp 8−19.

(2) Board on Agriculture, National Research Council. *Regulating Pesticides in Food*; National Academy Press: Washington, DC, 1987; p 209.

(3) Van de Poll, J. M.; Versluis-de Haan, G. G.; De Wilde, O. Determination of ethylenethiourea in water samples by gas chromatography with alkali flame ionization detection and mass spectrometric confirmation. *J. Chromatogr*. **1993**, *643*, 163−168.

(4) Kirk, J. R.; Mitchell, S. K. Risks and benefits associated with chlorine in the food industry. In *Water Chlorination: Environmental Impact and Health Effects*; Ann Arbor Science Publ., Inc.: Ann Arbor, MI, 1980; Vol. 3, p 283.

(5) Reina, L. D.; Fleming, H. P.; Humphries, E. G. Microbial control of cucumber hydrocooling water with chlorine dioxide. *J. Food Prot.* **1995**, *58*, 541−546.

(6) Dychdala, G. R. Chlorine and chlorine compounds. In *Disinfection, Sterilization, and Preservation*; Lea & Febiger: Philadelphia, PA, 1991; pp 157−182.
(7) Croue, J.-P.; Reckhow, D. A. Destruction of chlorination byproducts with sulfite. *Environ. Sci. Technol*. **1989**, *23*, 1412−1219.
(8) Alliger, H. *An Overall View of Chlorine Dioxide*; The Alcide Corp.: Farmingdale, NY, 1980.
(9) Lillard, H. S. Levels of chlorine and chlorine dioxide of equivalent bacterial effect in poultry processing water. *J. Food Sci*. **1979**, *44*, 1594−1597.
(10) Abdel-Rahman, M. S.; Couri, D.; Bull, R. J. Toxicity of chlorine dioxide in drinking water. *J. Environ. Pathol. Toxicol.* **1985**, *6*, 105−113.
(11) Junli, H.; Chao, Y.; Wang, X. Influence of chlorine dioxide on chloroform formation. *Environ. Chem*. **1994**, *13*, 466−473.
(12) *Standard Methods for Examination of Water and Wastewater*, 17th Ed.; American Public Health Association: New York, 1987; pp 298−300.
(13) Ahmad, N.; Guo, L.; Mandarakas, P.; Appleby, S. Determination of dithiocarbamate and its breakdown product ethylenethiourea in fruits and vegetables. *J. Assoc. Off. Anal. Chem*. **1995**, *78*, 1238−1243.
(14) Masschelein, W. J. Chlorine dioxide. In *Chemistry and Environmental Impact of Oxychlorine Compounds*; Ann Arbor Science Publishers Inc.: Ann Arbor, MI, 1979; pp 162−171.
(15) Morris, J. C. The chemistry of aqueous chlorine in relation to water chlorination. In *Water Chlorination. Environmental Impact and Health Effects*; Jolley, R. L., Ed.; Ann Arbor Science Publishers Inc.: Ann Arbor, MI, 1978; Vol. 1, pp 21−36.
(16) Junli, H.; Li, W.; Nanqi, R.; Fang, M.; Disinfection effect of chlorine dioxide on bacteria in water. *Water Res.* **1997**, *31*, 607−613.
(17) Engst, R.; Schnaak, W. Residues of dithiocarbamate fungicides and their metabolites on plant foods. *Residue Rev.* **1974**, *52*, 45−67.

Received for review March 12, 2002. Revised manuscript received May 27, 2002. Accepted May 28, 2002. Acknowledgment is made to the Michigan State University Agricultural Experimental Station and Michigan Apple Committee for their support of this research. Use of a commercial brand or company name is not an endorsement by Michigan State University.

JF020307C