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# Influence of Phosphate and Copper on Reductive Dechlorination of Thiobencarb in California Rice Field Soils

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The potential for reductive dechlorination of the herbicide thiobencarb (TB) by microbes and its prevention in saturated anaerobic rice field soils was examined in laboratory microcosms. TB is effective in controlling both annual grasses and broadleaf weeds. In anoxic microcosms, TB was effectively degraded within 30 days to its dechlorinated product, deschlorothiobencarb (DTB), in two Sacramento Valley rice field soils. TB dechlorination, and subsequent degradation, followed pseudo-zero- (lag phase) and first-order (degradation phase) kinetics. Logistic regression analysis ($r^2 > 0.841$) produced a half-life ($t_{1/2}$) in nonsterile soils ranging from 10 to 15 days, which was also observed when microcosms were amended with low concentrations (<3 mg $L^{-1}$) of copper ($Cu^{2+}$; as the fungicides $Cu(OH)_2$ and $CuSO_4 \cdot 5H_2O$). High $Cu^{2+}$ concentrations (>40 mg $L^{-1}$) were added to the microcosms to determine if copper toxicity to dechlorinating microbes is concentration dependent within the range used. After 30 days, the low-copper-amended soils closely resembled the nonsterile experiments to which no $Cu^{2+}$ was added while the high-copper-amended microcosms were similar to the sterile experiment. Microcosms were also separately amended with 5.7 g $L^{-1}$ phosphate ($PO_4^{2-}$; as $KH_2PO_4$), a nutrient regularly applied to rice fields. Phosphate-amended experiments also showed TB degradation, but no DTB formation, indicating the phosphate played a role, possibly as a microbial inhibitor or an alternative electron acceptor, in limiting the dechlorination of TB. In summary, TB dechlorination was inhibited at high $Cu(OH)_2$, $CuSO_4 \cdot 5H_2O$, and $KH_2PO_4$ concentrations.

**KEYWORDS: Microcosms; herbicide; fungicide; anaerobic; half-life**

## INTRODUCTION

Thiobencarb (TB, *S*-4-chlorobenzyl diethylthiocarbamate, trade names Bolero and Saturn) is an herbicide commonly used in rice fields worldwide to control annual grasses, such as barnyard grass (*Echinochloa* spp.) and certain broadleaf weeds (*1*). It is a highly effective, nonpersistent, systemic pre-emergence herbicide that both interferes with protein synthesis and inhibits photosynthesis (*2*). In California, approximately 270 000 kg were applied to rice fields in 2002 (*3*).

Previous studies have shown that TB distributes extensively between the air, water, biota, and soil (*4*, *5*). However, since TB possesses limited water solubility of 30 mg/L (*2*), it is most prevalent in soils where, via degradation, it serves as a carbon source for microbes (*4*). Reductive dechlorination of the herbicide is problematic because the product, deschlorothiobencarb (DTB, *S*-benzyl diethylthiocarbamate), causes dwarfing of rice plants with subsequent loss in yields. Known as delayed phytotoxicity syndrome (DPS), it was first observed three decades ago in Japanese rice fields and more recently both in Louisiana and California (*6*, *7*). Studies by Tatsuyama et al. (*8*), Moon and Kuwatsuka (*9*−*12*), and Nakamura et al. (*13*) have determined that TB dechlorination is microbially mediated and that some soil properties can influence the process.

Although several reports have determined that TB dechlorination is microbial mediated, none of them examined measures to prevent the process from occurring. The primary focus of this study, therefore, was to evaluate if copper and phosphate can prevent dechlorination of TB in rice field soils. Thus, in addition to examining the influence of some soil conditions (wet vs dry and physical−chemical properties) on reductive dechlorination of TB, the effects of low and high fungicidal copper ($Cu^{2+}$, as a metabolic inhibitor) and phosphate ($PO_4^{2-}$ as a microbial inhibitor or alternative metabolic terminal electron acceptor) on TB degradation were also examined in two anaerobic Sacramento Valley rice field soils. The pseudo-first-order and logistic degradation half-lives for TB were measured and discussed in this report.

## MATERIALS AND METHODS

**Chemicals and Soils.** Technical-grade TB and DTB were supplied gratis by Valent (Fresno, CA) and Kumiai Chemical Industry, Co., Ltd. (Tokyo, Japan), respectively. High-purity molinate was obtained from Syngenta (Richmond, CA). Analytical-grade cuperic hydroxide

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**Table 1.** Physical–Chemical Characteristics of M and B Soils[a]

| soil parameter | B | M | method |
|---|---|---|---|
| sand (%) | 29 | 33 | particle size analysis |
| silt (%) | 45 | 45 | |
| clay (%) | 26 | 22 | |
| electric conductivity (dS/m) | 0.200 | 0.406 | EC meter |
| pH | 4.80 | 4.94 | pH meter |
| organic matter (%) | 2.34 | 2.21 | Walkley-Black |
| carbon (%) | 1.60 | 1.43 | Carlo Erba Combustion |
| nitrogen (%) | 0.10 | 0.13 | |
| $NH_4$–N (mg/kg) | 23.1 | 20.4 | |
| $NO_3$–N (mg/kg) | <0.10 | 3.18 | |
| total Mn (mg/kg) | 421 | 547 | microwave digestion followed by atomic absorption spectroscopy and inductively coupled plasma atomic emission spectroscopy (ICP-AES) |
| total Cu (mg/kg) | 67.8 | 38.2 | |
| total Zn (mg/kg) | 73.4 | 66.4 | |
| total Fe (g/kg) | 28.7 | 21.9 | |
| total S (mg/kg) | 27.2 | 32.3 | ICP-AES |
| exchangeable $PO_4$–P (mg/kg) | 5.55 | 3.75 | Bray |
| exchangeable K (mg/kg) | 88.0 | 72.6 | equilibrium extraction followed by AES |
| exchangeable Na (mg/kg) | 27.0 | 44.2 | |
| exchangeable Ca (meq/100 g) | 7.50 | 6.30 | |
| exchangeable Mg (meq/100 g) | 4.40 | 3.50 | |

[a] Concentration or percent distribution of some important soil parameters for M and B soils. The method in which these parameters were obtained is also presented.

($Cu(OH)_2$), cuperic sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$), and potassium dihydrogen phosphate ($KH_2PO_4$) were purchased from Alfa Aesar (Ward Hill, MA), Fisher Scientific (Springfield, NJ), and J. T. Baker (Phillipsburg, NJ), respectively.

Rice field soils were collected from two farms in the Sacramento Valley of California. The farms, Baggett (soil B) and Mathews (soil M), are located in Yuba county, and both have soils characterized as San Joaquin Series fine mixed thermic Abruptic Durixeralfs. Both farms have historically experienced DPS. Soils were collected approximately 10 feet from the perimeter of each of the fields' four boundaries, as well as from their center. Samples were collected using inverted 50-mL falcon tubes with an air−water bypass valve. They were filled with rice water from the flooded field and pushed into the soil. As soil filled the tube, the air and water in the tube were displaced via the bypass valve. The valve was removed and the tube was sealed under the water surface to prevent exposure of the anaerobic soil to oxygen. Samples (40) from each field were pooled and homogenized in a conventional blender under $N_2$ gas in an inflatable glovebag ($I^2R$, Cheltenham, PA) in the laboratory. The homogenized soils were stored at 4 °C under $N_2$ headspace in saturation airtight plastic containers until used.

**Soil Characterization.** A number of physical−chemical properties for B and M soils were characterized by the Agriculture and Natural Resources (ANR) Analytical Laboratory at UC Davis; both the methods and results are provided in Table 1. A detailed description of the methods can be found in Schmelzer et al. (*14*).

**Soil Microcosms.** TB degradation, and subsequent formation of DTB, was monitored using soil microcosms maintained according to the experimental procedures of Kaspar and Tiedje (*15*), with slight modifications. Briefly, 6 g of wet soil (water content ∼ 30%) and 12 mL of untreated well water (Placer County, CA) were placed in clear 60-mL serum bottles and sealed under $N_2$ gas with butyl stoppers and aluminum crimp caps (Wheaton, Millville, NJ). The soil:water ratio (∼1:3.5) was chosen to mimic flooded rice field conditions. The bottles were placed in a temperature-controlled oven at 30 ± 1 °C in the dark to prevent photolysis of the herbicide. The selected temperature is typical of daytime highs in California rice field soils (*16, 17*). They were allowed to incubate for 7 days prior to addition of 97 $\mu$M analytical-grade TB or any of the subsequent treatments (addition of copper or phosphate).

Low copper ($Cu^{2+}$) treatments involved adding 0.1 mL to the microcosms from high stock solutions of 2.12 mM $CuSO_4 \cdot 5H_2O$ and 3.39 mM $Cu(OH)_2$ to correspond with a final $Cu^{2+}$ concentration of 2.42 and 1.55 mg $L^{-1}$ (15.3 $\mu$M $CuSO_4 \cdot 5H_2O$ and 24.4 $\mu$M $Cu(OH)_2$), respectively. Similarly, the high-copper-amended microcosms were exposed to 1 mL of a 10.1 and 19.1 mM stock solutions, which correspond to 43 and 82 mg $L^{-1}$ $Cu^{2+}$ for $CuSO_4 \cdot 5H_2O$ and $Cu(OH)_2$, respectively. Final concentration in the high copper-amended microcosms were 0.68 mM for $CuSO_4 \cdot 5H_2O$ and 1.29 mM for $Cu(OH)_2$. The field equivalent rates, with a 1.5-cm rice water flood depth, for the low $CuSO_4 \cdot 5H_2O$ (herein referred to as $CuSO_4$) and $Cu(OH)_2$ microcosm experiments were 0.15 and 0.23 kg active ingredient (a.i.)/ha, while it was 6.5 and 12.27 kg a.i./ha for the high concentration studies, respectively. The phosphate-amended experiment involved adding 1 mL of $KH_2PO_4$ from a 0.88 M stock solution to the microcosms to produce a final phosphate ($PO_4^{2-}$) concentration of 5.7 g $L^{-1}$ (60 mM $KH_2PO_4$), field equivalent rate of 849 kg a.i./ha. Stock solutions were prepared in ultrapure water. All amendments were conducted after an initial 7-day incubation period and run in triplicate ($n = 3$ at each sampling point) including the controls that had TB in flooded, but autoclaved, soil.

Prior to extraction of TB and DTB, the oxidation−reduction potential of each serum bottle was measured and recorded using an Orion model 250A ion-selective electrode meter (Beverly, MA) with an Accumet redox probe (Fisher Scientific, Springfield, NJ). Experimental procedures were conducted in inflatable glovebags under constant $N_2$ gas flow to maintain anaerobic conditions. The same procedures were used to prepare microcosms for soils that had been dried after storage.

**Chemical Extraction.** TB and DTB were extracted using the methanol/hexane procedure of Schmelzer et al. (*14*). Prior to extraction, approximately 27.0 $\mu$M of molinate (as an analytical surrogate) were added to each microcosm. Extraction involved adding 4 mL of methanol to the serum bottle, which was vortexed briefly then shaken for 10 min on a wrist-action shaker. Then, 3 mL of hexane were added to the bottle, which was again vortexed, shaken, and centrifuged at 1000*g* for 10 min. The supernatant was collected, and samples were extracted twice more with 4 mL each of hexane. The supernatant from each hexane extraction was pooled and concentrated to 5 mL under $N_2$ gas.

**Chemical Analysis.** The concentrations of TB and DTB from each microcosm were determined using a Hewlett-Packard model 5890 gas chromatograph with a model 5971 mass-selective detector (MSD) from (Palo Alto, CA). A Pheonomenex (Torrance, CA) ZB-50 capillary column (30m × 0.25 mm inside diameter (i.d.), 25-mm film thickness) was used with He as the carrier gas. Samples were introduced to the GC-MSD in splitless mode via an autosampler (Hewlett-Packard model 7673). The GC had an initial injector temperature of 250 °C, detector temperature of 280 °C, and a ramped oven temperature (100 °C at 1 min followed by 20 °C/min to 270 °C). The detector was operated in selective-ion mode: 72, 100, 125, and 257 *m*/*z* for TB; 72, 100, and 233 *m*/*z* for DTB; and 55, 98, 126, and 187 *m*/*z* for molinate. Quantitation was via standard reference calibration curves with molinate

**Table 2.** Thiobencarb Degradation in M and B Soil Microcosms with Time and Calculated Pseudo-First-Order and Logistic Half-Life ($t_{1/2}$) Values; $t_{1/2}$ Results for $CuSO_4 \cdot 5H_2O$ (Shown as $CuSO_4$) and $Cu(OH)_2$ Treatments

| | | thiobencarb remaining (%) at days | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| soil | treatment | 10 | 15 | 21 | 30 | $t_{1/2}$ logistic (days) | $r^2$ logistic | $t_{1/2}$ pseudo-first order (days) | $r^2$ pseudo-first order |
| M | nonsterile control | 94 | 60 | 8 | 5 | 14.31 | 0.841 | 12.73 | 0.912 |
| M (dry) | nonsterile control | 96 | 68 | 38 | 5 | 16.83 | 0.852 | 12.35 | 0.822 |
| M | $CuSO_4$ at 15.3 $\mu$M | 71 | 42 | 8 | | 12.76 | 0.987 | 12.41 | 0.943 |
| M | $Cu(OH)_2$ at 24.4 $\mu$M | 74 | 68 | 11 | | 14.16 | 0.882 | 12.94 | 0.826 |
| B | nonsterile control | 67 | 10 | 10 | 5 | 10.88 | 0.848 | 13.30 | 0.922 |
| B | $CuSO_4$ at 15.3 $\mu$M | 31 | 11 | 4 | | 11.39 | 0.947 | 12.92 | 0.992 |
| B | $Cu(OH)_2$ at 24.4 $\mu$M | 26 | 12 | 8 | | 10.20 | 0.911 | 15.34 | 0.947 |

**Table 3.** Statistical Analysis of the Logistic Decay Curves for the Untreated, $CuSO_4 \cdot 5H_2O$ (Shown as $CuSO_4$), $Cu(OH)_2$, and $KH_2PO_4$ Treatments of M and B Soils

| soil | treatment | P value | significance[a] |
|---|---|---|---|
| M | nonsterile control | | a |
| M (dry) | nonsterile control | 0.4129 | a |
| M | $CuSO_4$ at 15.3 $\mu$M | 0.6983 | a |
| M | $Cu(OH)_2$ at 24.4 $\mu$M | 0.6260 | a |
| M | $CuSO_4$ at 0.68 mM | 0.0002 | b |
| M | $Cu(OH)_2$ at 1.29 mM | 0.0002 | b |
| M | $KH_2PO_4$ at 60 mM | 0.0015 | b |
| B | nonsterile control | | a |
| B | $CuSO_4$ at 15.3 $\mu$M | 0.7801 | a |
| B | $Cu(OH)_2$ at 24.4 $\mu$M | 0.9164 | a |
| B | $CuSO_4$ at 0.68 mM | 0.0174 | b |
| B | $Cu(OH)_2$ at 1.29 mM | 0.0261 | b |
| B | $KH_2PO_4$ at 60 mM | 0.0027 | b |

[a] a and b are groups in which the slopes of the logistic regression are not significantly different ($p > 0.05$).

as the internal standard. Detection limits were 0.84 pM for TB, 0.48 pM for DTB, and 0.73 pM for molinate as determined by Schmelzer et al. (*14*).

**Statistical Analysis** The half-life values ($t_{1/2}$) reported in this study were calculated using two models. The first was the pseudo-first-order model that has been widely used to calculate microbial $t_{1/2}$ values for numerous organic compounds (*18*, *19*). The second was the logistic model that is useful for investigation of relationships between proportions and continuous variables (*20, 21*). The proportion in this study is the TB concentration loss in relation to its initial concentration, and the continuous variable is time. Table 2 shows that the logistic model fits the data quite well ($r^2 > 0.841$). Microsoft Excel 2002 and Sigmaplot 2000 (version 6.0) graphing software were used to fit the data to the models, and statistical analysis software (SAS Version 8) was used to determine if the slopes of the logistic fit curves were statistically similar or different (Table 3).

## RESULTS

**Thiobencarb Dechlorination in Soils.** Figure 1 shows the degradation of TB and subsequent formation of DTB in flooded nonsterile and sterile microcosms for the M soils. TB degradation did not appreciably occur in the sterile soils, while in the nonsterile soils it readily occurred with subsequent formation of DTB. The measured oxidation−reduction potential (not shown) confirmed that the vials were highly anaerobic, $> -200$ mV after the 7-day incubation period. **Figure 1** also shows that continuously wet soil (anaerobic) produced TB degradation and DTB formation patterns similar to the soils that had been dried during storage (**Table 2**). The data further show that, although the soil was dried, a 7-day incubation time was sufficient for the dry soils to become anoxic in flooded conditions and provide a suitable environment for the revitalization of microbes that were originally present in the rice fields. Similar TB degradation and DTB formation trends were observed for B soils.

**Thiobencarb Degradation Half-Life.** A clear trend within all the incubations was a lag phase from 0 to 10 days (**Figure 1**). **Table 2** shows this trend (with ∼95% TB remaining in the nonsterile M soils after 10 days of incubation). Rapid degradation of TB followed between 10 and 20 d. Examination of the TB degradation curves (**Figure 1**) indicates two reaction processes: presence of a zero-order or "saturated" system between 0 and 10 days and pseudo-first-order decay after 10 days. The results from the pseudo-first-order exponential decay fitting are presented in **Table 2**. The lag phase was shorter in the M soils to which low concentrations of $CuSO_4$ and $Cu(OH)_2$ had been added, as 20% less TB remained in the copper-treated microcosms at day 10 as compared to the nonsterile experiment (**Table 2**). In general, the B soils had a shorter lag phase compared to the nonsterile and copper-amended M soils. Similar lag phases were observed by Moon and Kuwatsuka (*10*) for thiobencarb and Bunge et al. (*22*) when they studied the reductive dehalogenation of 1,2,4-trichlorodibenzo-*p*-dioxin to the dechlorinated 1,3-dichlorodibenzo-*p*-dioxin in freshwater sediments by *Dehalococcoides* spp.

An important parameter extrapolated from the TB microbial degradation studies over time is the $t_{1/2}$ of the herbicide. The results show that degradation of TB in the presence of a low copper concentration closely follows the degradation $t_{1/2}$ values of the nonsterile soils. This indicates that the low copper concentration treatment ($<3$ mg $L^{-1}$ of $Cu^{2+}$) did not inhibit TB dechlorination. Similar $t_{1/2}$ results were obtained when the data was modeled after the logistic decay function. The primary use for such a model was to determine the $t_{1/2}$ inclusive of the zero-order decay data points (0−10 days) that had been omitted in the pseudo-first-order decay model. The calculated logistic $t_{1/2}$ values for the M soils showed that the pseudo-first-order results (**Table 2**) were within ±2 days (12.76−16.83 days) of the untreated nonsterile wet soil (14.31 days). The first-order calculations support the logistic results, as the $t_{1/2}$ values are very similar for the M soils. The $t_{1/2}$ values for TB in B soils were lower in general, according to the logistic results when compared to the M soils although the two soils do not vary greatly in physical-chemical properties (**Table 1**). In both soils, TB would be completely degraded within 30 days as predicted by the $t_{1/2}$ results, regardless of the model used.

**Effect of High Copper and Phosphate Concentrations on TB Dechlorination.** Since a low copper concentration, in relation to the soil background total copper concentrations (38−68 mg/kg), did not inhibit the microbes responsible for TB dechlorination, we examined the effect of a high copper concentration on the process. **Figures 2** and **3** show TB in M and B soils treated with a high copper concentration. The addition of high levels of copper (0.68 and 1.29 mM $CuSO_4$ and $Cu(OH)_2$ to produce 43 and 82 mg $L^{-1}$ $Cu^{2+}$ treatment, respectively) to the M and B soil microcosms revealed contrast-

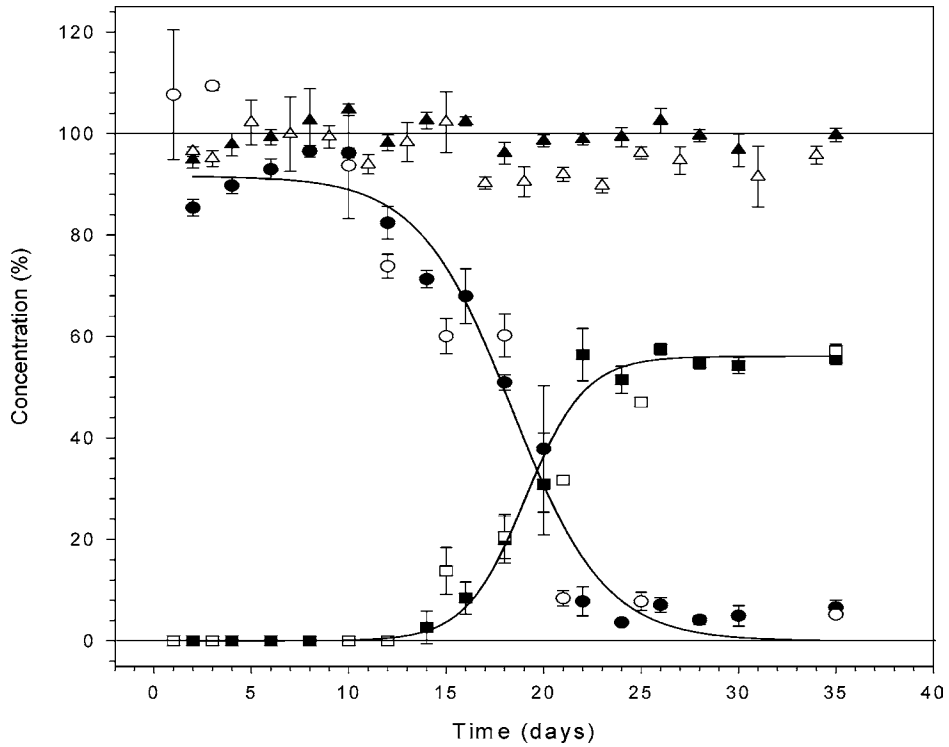


**Figure 1.** Percent degradation of TB using nonsterile dried (●) and permanently wet (○) M soil and the subsequent formation of DTB (dried = ■, permanently wet = □). Similar degradation of TB and DTB was obtained for soil B (not shown). The sterile wet (Δ) and dried (▲) soils show insignificant loss of TB over the 35-day sampling period. The solid lines show the relative logistic degradation and formation for TB and DTB in the permanently wet soil, respectively. Points represent means, and bars represent standard error; $n = 3$.

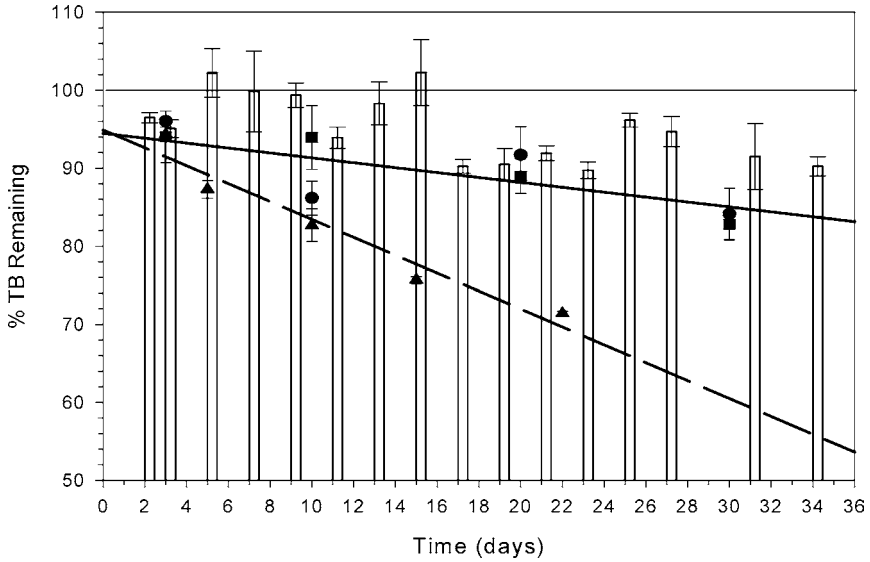


**Figure 2.** Degradation of TB in sterilized (vertical bars) and nonsterilized (points) M soils with 1.29 mM $Cu(OH)_2$ (●), 0.68 mM $CuSO_4 \cdot 5H_2O$ (■), and 60 mM of $KH_2PO_4$ (▲). Points represent means, and error bars represent standard error; $n = 3$. The lines represent the relative linear regression for the copper (solid line) and $KH_2PO_4$ (dashed line) treatment.

ing results to that observed for the low concentration treatment. The loss of TB in the microcosms with the high copper concentration closely followed the degradation pattern of the sterile controls with no added copper in which no DTB formation was observed over 30 days.

The effects of $PO_4^{2-}$ (60 mM $KH_2PO_4$) on the degradation of TB over 22 days are presented in **Figure 2** and **3**. The phosphate-amended microcosms had TB losses of 22 and 37% in the M and B soils, respectively. However, no DTB was formed within 22 days, sufficient time for the dechlorinated chemical species to become detectable (**Figure 1**).

Statistical analysis (**Table 3**) of the logistic degradation curves showed that the low concentration copper-treated microcosms were not significantly different from the nonsterile untreated microcosms for M and B soils ($p > 0.05$). In contrast, the high copper and phosphate concentrations were significantly different from the nonsterile untreated B and M soils ($p < 0.05$).

## DISCUSSION

The results show that TB dechlorination occurs readily within 30 days in Sacramento Valley rice field soils and that it is primarily driven by anaerobic microbes (**Figure 1**). The

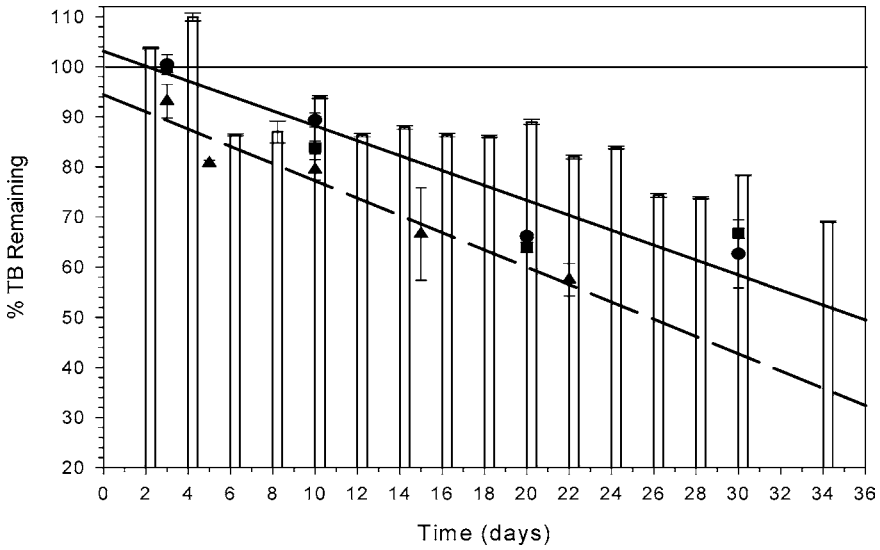


**Figure 3.** Degradation of TB in sterilized (vertical bars) and nonsterilized (points) B soils with 1.29 mM $Cu(OH)_2$ (●), 0.68 mM $CuSO_4 \cdot 5H_2O$ (■), and 60 mM of $KH_2PO_4$ (▲). Points represent means, and error bars represent standard error; $n = 3$. The lines represent the relative linear regression for the copper (solid line) and $KH_2PO_4$ (dashed line) treatment.

subsequent formation of DTB, which is toxic to rice plants (*23*), was also evident within a short time period (15 days). Additionally, wet and dry cycles, present during the growing season and harvest, respectively, did not impair the microbes responsible for TB dechlorination in M and B soils (**Figure 1**). This indicates that the responsible microbial populations are capable of surviving through aerobic dry soil conditions, possibly by spore formation. Spore-forming obligate anaerobes closely resembling the gram-positive *Clostridia* bacterial group have been identified in rice field soils (*24*). Other studies have confirmed that the microbes responsible for TB dechlorination are a type of bacteria (*11*). For instance, Abe et al. (*25*) found that *Coryneform* bacteria are capable of degrading thiobencarb. We are currently attempting to identify the microbial species responsible for TB dechlorination in California rice field soils using gene-sequencing methods similar to those of Akasaka et al. (*24*).

The $t_{1/2}$ calculations reveal that TB dechlorination occurs within a short time period under anaerobic rice field soil conditions. A recent study showed similar results, with $t_{1/2}$ values around 20 days (*14*). The findings may have important implications when considering the role of TB in rice fields; it may be transformed to the dechlorinated product within a shorter time period than previously thought. For instance, the California Department of Pesticide Regulation requires a rice water holding time of 24−30 days, depending on the compound, to allow for sorption and dissipation of aqueous herbicides. This study shows that this time period is adequate when considering the dechlorination of TB but not the case for DTB since it becomes present many days after the application time of the parent compound (15 days). According to a recent study by Schmelzer et al. (*14*), the dechlorinated compound is persistent for more than 90 days after the TB application.

We attempted to control the dechlorination of TB by inhibiting the microbes responsible with copper-based ($Cu^{2+}$) inhibitors. Copper sulfate has been shown to be an effective antimicrobial agent (*26*), while $Cu(OH)_2$ has been historically used as a fungicide (*27*). The amendment of 0.68 and 1.29 mM $CuSO_4$ and $Cu(OH)_2$, respectively, to the microcosms (to provide 43 and 82 mg $L^{-1}$ of $Cu^{2+}$, respectively) resulted in no significant difference between the dechlorination of TB in test soils vs sterile soils. Thus, the dechlorination of TB was halted, indicating that high copper concentrations inhibit microbes responsible for DTB formation. In contrast, addition of low copper concentrations (15.3 $\mu$M $CuSO_4$ and 24.4 $\mu$M $Cu(OH)_2$, to produce 2.42 and 1.55 mg $L^{-1}$ of $Cu^{2+}$, respectively) to the two soils did not inhibit TB dechlorination. This was anticipated, as the background soil copper concentration (38−68 mg/kg) was significantly higher than the applied amount. The high soil background copper could have resulted from past use of Kocide-soaked rice seeds. Kocide (Griffen L.L.C., Valdosta, GA), whose active ingredient is $Cu(OH)_2$, has been historically used as a rice seed fungicide. The copper from the seed husks may have desorbed into the soil and accumulated slowly over time.

TenBrook et al. (*28*) found a positive correlation between high total copper concentration, high clay content, and low sand content with reduced DPS formation in several Sacramento Valley soils. These soils contained about 20 mg/kg of background copper. In this study, the background copper concentrations in the soils were 2−3 times higher than reported in TenBrook et al. (*28*), but still TB dechlorination occurred (**Table 2**). Therefore, total soil copper cannot independently account for reduced DPS formation in rice field soils treated with TB. However, the availability of copper in the soil−water interphase could. For instance, in this study the low copper amendments did not inhibit DPS formation in the soils because the copper could be readily adsorbed but, at high concentrations, the metal ions may saturate the soil sorption sites and therefore be available in solution. This "bioavailable" copper may now inhibit those microbes that use chlorine from TB as a terminal electron acceptor.

The mechanism of copper availability may also explain why high clay and low sand content result in reduced DPS formation as found by TenBrook et al. (*28*). High clay soils can hold greater amounts of water and cations and thus act as a bilateral copper sink by having available copper in the water interphase, given low pH conditions (*29*), as opposed to sandy soils that do not hold water. Choudhury and Khanif (*29*) found that increased copper concentrations resulted in greater amounts of the element in the equilibrium solution of sorption experiments. They also found that copper adsorption was positively correlated with pH due to an increase in the monovalent $CuOH^+$ ions at high pH. This type of copper is known to be adsorbed (or precipitate out of solution) in much greater quantities than the

divalent $Cu^{2+}$ ions (*30*) that would be present in low pH conditions. Thus, at the high concentrations of $Cu(OH)_2$ and $CuSO_4$ applied to the microcosms in this study and given the low pH soils used (4.8−4.9) and in that (4.8−5.9) by TenBrook et al. (*28*), copper would have been in the divalent form due to the acidic conditions and thus readily available. The specific mechanism of copper toxicity to microbes, according to the literature as it was beyond the scope of this study, is not fully understood, but studies have shown that copper ions can disrupt the plasma fatty acid composition of microbial cell walls (*31*) and that their association with DNA could contribute to genotoxicity (*32*).

The phosphate microcosm results showed a similar trend to that observed with the high copper concentration: no DTB formation over 21 days. However, TB degradation did occur over the 30-day study period (55 and 40% TB degradation for B and M soils, respectively). The results of this study are consistent with other work that showed that microbial activity was positively correlated with phosphate concentration (*9*) and it could be utilized by anaerobic, denitrifying, phosphorus-accumulating microorganisms (*33*). These findings suggest that phosphate fills a microbial niche similar to that of the TB-derived chlorine but it could also act as a microbial inhibitor given the high concentrations used in this study (277 kg/ha phosphorus) as compared to normal application rates of 20 kg/ha phosphorus to rice field soils (*34*). Microbial inhibition by phosphate is supported by Conrad et al. (*35*) who found that methanogenesis can be inhibited at these high concentrations (50 mM $KH_2PO_4$). Further, phosphate is not the most efficient electron acceptor, but nevertheless, it seemed to play an important role in preventing dechlorination. Additional TB studies using field application rate phosphate concentrations need to be conducted under laboratory conditions to determine if the same benefits, as observed in this study, are obtainable.

Overall, this investigation has shown that TB dechlorination in California rice field soils is indeed a microbially mediated process but can be eliminated by adding microbial inhibitors. The dechlorination can be controlled using high copper amendments or high nutrient concentrations (acting as an inhibitor or as an alternative electron acceptor). Although, the high concentrations used in this study are significantly higher than normal application rates, this study showed that background soil concentrations and normal application rates of copper are not sufficient to eliminate the dechlorination of TB in California rice field soils. The microbes are able to survive wet−dry cycles and are resistant to low copper amendments as well as capable of adapting to high background soil copper concentrations over time. Further, it should be noted that the high application rates used here could affect other microbial communities that are nonessential to the dechlorination process but essential for other important microprocesses in soils such as organic matter formation. We are currently attempting to identify the microbial species in the same rice field soils by DNA extraction followed by 16s rRNA gene sequencing.

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