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Comparative Toxicity of Thiobencarb and Deschlorothiobencarb to Rice (*Oryza sativa*)

A. J. Palumbo, P. L. TenBrook, A. Phipps, R. S. Tjeerdema

Department of Environmental Toxicology, College of Agricultural and Environmental Sciences, University of California, One Shields Avenue, Davis, CA 95616-8588, USA

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Thiobencarb (*S*-4-chlorobenzyl *N,N*-diethylthiocarbamate; TB) is an herbicide used to control certain grasses, sedges, and broad leaf weeds in rice fields. It is a systemic, pre-emergence herbicide that acts by inhibiting shoots of emerging seedlings (U.S. EPA, 1997). However, TB can also potentially injure the desired crop. After using TB, rice growers have historically observed dwarfing, dark-green leaves, deformities, excessive tillering, and plant death. These symptoms characterize delayed phytotoxicty syndrome (DPS). Crop injury from DPS reduces rice yield and has been a serious problem for growers in Northern California, Louisiana, Missouri and Japan (Kendig and Johnson, 1997; Groth *et al.*, 1999; C & P Press, 2002).

In the early 1990s, as northern California rice farmers began to use the granular form of TB they also began to experience DPS. This formulation is applied to flooded rice fields approximately 10-14 d after seed application, and the fields are maintained flooded (10 cm depth) for another 28 d. Currently, the only suggested course of action for a DPS-affected field is drainage to re-oxygenate the soil (Groth *et al.*, 1999; C & P Press, 2002). However, this is undesirable, as it causes nitrogen loss, increased susceptibility to weeds and diseases, and negative impacts to fish and wildlife (Groth *et al.*, 1999). These environmental concerns prompted a ban on use of the granular form in northern California (C & P Press, 2002).

Early investigations into the cause of DPS found detectable concentrations of deschlorothiobencarb (*S*-benzyl *N,N*-diethylthiocarbamate; DTB), an anaerobic microbial degradation product of TB (Figure 1), in soils where DPS was observed (Isiwaka *et al.*, 1980). A bioassay using rice seedlings grown in soils spiked with either TB or DTB found that DTB caused height reductions at lower concentrations than TB, indicating that DTB may cause DPS (Tatsuyama *et al.*, 1981). Recently, Chen (2002) found DTB also caused rice dwarfing at lower concentrations than TB in both agar and Louisiana rice field soils.

Although DTB is implicated as the cause of DPS, there is a lack of information in the literature to support this theory. Therefore, the objective of this research was to determine if DTB produces DPS-like symptoms in a standardized non-soil

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Correspondence to: A. J. Palumbo

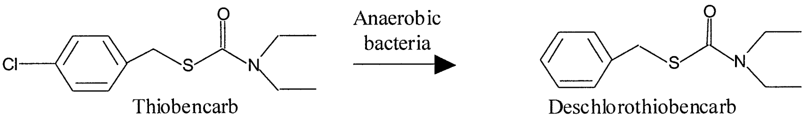

Figure 1. Microbial dechlorination of thiobencarb.

medium, and further to determine if DTB can produce DPS at field-relevant concentrations by comparing the no observable effect concentration (NOEC), and the 50% inhibition concentration (IC_{50}), values for TB and DTB.

MATERIALS AND METHODS

To determine absolute differences in toxicity, rice plants (*Oryza sativa,* L. variety M202) were grown from seeds in a hydroponic culture system that minimized complications in dosing as well as biotic and abiotic degradation. Seeds were sprouted by placing a single seed in a 25-mL test tube with 15 mL (10 cm depth) of de-ionized water; the bottom 5 cm of the tube was covered with aluminum foil to limit root light exposure. The plants were kept in a controlled-environment chamber throughout the experiment (14 hr, 30°C day/10 hr, 16°C night; relative humidity: 30% day/80% night; maximum photosynthetically active radiation = 800 nm). After 7 d the de-ionized water was replaced by a nutrient solution of 1x Hoagland's No. 2 Basal Salt Mixture, pH 6.0 ± 0.1 (Sigma, St. Louis, MO). Exposures to TB and DTB began within the time period DTB is expected to appear in a field (6 d after TB application; plants at 16-d old, 4-leaf stage, in a DPS conducive soil with 2% straw; Schmelzer *pers. comm.,* 2002). Chemical exposure was an 8-d static–renewal with dosing solutions replaced every 48 hr to maintain relatively stable concentrations. Six solutions of TB (technical grade; Valent, Walnut Creek, CA) were prepared based on range finding experiments to optimally bracket the IC_{50}, between 0.19 to 40 μM in Hoagland's solution (see above), while those for DTB (Kumiai Chemical Co., Tokyo, Japan,) were prepared similarly between 0.035 to 14 μM. A total of three bioassays were performed for each agent, with each of the six concentrations being replicated four times per bioassay. Controls contained plants in Hoagland's solution only.

Exposure concentrations were confirmed via hexane extraction followed by gas chromatography-mass spectrometry (GC-MS), per previously developed methods (Schmelzer *pers. comm.,* 2002). Briefly, 1 mL of methanol (pesticide grade) and 20 ng molinate (as a surrogate standard) were mixed into to 2.5 mL samples of the solutions before extracting 3 times with 4 mL of hexane. The extracts were dried over anhyd. magnesium sulfate and concentrated under N_2. Analyses were performed on 1 μL of each extract, in duplicate, via GC-MS. The GC was equipped with a ZB-50 capillary column (30 m x 0.25 mm id, 25 μ film thickness). Helium (0.6 mL/min) was the carrier gas, the injector temperature was 250°C, and the detector temperature was 280°C. The GC oven was initially set at 100°C for 1 min, then increased at 20°C/min to 270°C and held for 1 min. The MS was operated in the selective ion-monitoring mode.

Test solutions were analyzed before and after the 48-hr static-renewal period and the concentrations of TB and DTB were found to have decreased. Therefore, the average concentrations during the 48-hr time period for each of the 3 tests were used for calculation of NOECs and IC_{50}s for the individual tests. These 3 concentrations were averaged to construct a single dose-response curve for each compound and to calculate the mean NOEC and IC_{50} values for all data (Table 1).

Preliminary tests showed that dwarfing was the major symptom observed in hydroponic exposures to both TB and DTB. Therefore, shoot growth during exposure was selected as the toxic endpoint. Shoot heights were recorded at the beginning and end of the 8-d exposure period and change in height was used to develop a dose-response relationship. Concentrations of TB or DTB causing 25% and 50% reductions in shoot height compared to controls (IC_{25} and IC_{50}, respectively) were calculated using linear interpolation (ToxCalc v. 5.020, Tidepool Scientific Software, McKinleyville, CA). The IC_{25} was used to estimate the NOEC (US EPA, 1991). NOECs and IC_{50}s were compared with unpaired t-tests with Welch correction (Instat v. 3.05 GraphPad Software Inc., San Diego, CA).

RESULTS AND DISCUSSION

The graded dose-response curves (Figure 2) illustrate a clear trend of decreasing plant growth with increasing concentration of either TB or DTB, with DTB effects occurring at lower concentrations. At higher concentrations signs of plant mortality, such as yellowing and browning of leaves, were also observed (data not shown). The observed effects resembled those of DPS and support the hypothesis that DTB is the cause. Some DPS-like symptoms, such as deformed and dark green leaves, were not observed in these experiments. However, Chen (2002) did report dark green foliage from exposure to TB or DTB in Louisiana soils.

The NOEC and IC_{50} values (Table 2) show that DTB is approximately 10 times more toxic to rice than the parent herbicide. This is similar to ratios of IC_{50}'s extrapolated from Tatsuyama *et al.* (1981) and Chen (2002, both using soil), as well as IC_{50}s reported by Chen (2002, using agar; Table 3). Considering that the studies were conducted in three different media types, the results are fairly

Table 1. Nominal and measured test concentrations.

Thiobencarb (μM)			Deschlorothiobencarb (μM)		
Nominal	Average	Standard Deviation	Nominal	Average	Standard Deviation
38.8	32.6	4.6	14.2	11.3	0.4
19.4	9.85	2.9	5.6	3.9	0.1
3.9	1.9	0.5	2.2	1.5	0.2
1.9	0.89	0.35	0.6	0.4	0.02
0.39	0.32	0.18	0.22	0.16	0.02
0.19	0.25	0.16	0.04	0.04	0.01

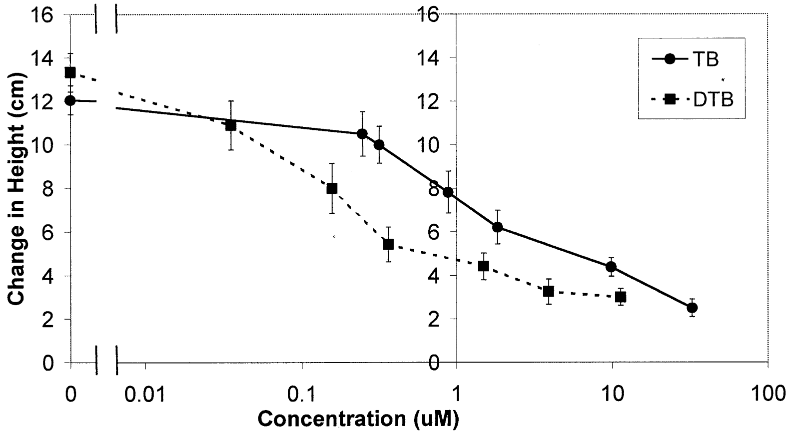

Figure 2. Inhibition of rice growth by thiobencarb and deschlorothiobencarb (bars represent mean ± SE, n =10 - 12).

consistent, although the IC_{50} values for the soil experiments were considerably larger than those derived from the hydroponic and agar tests. This is expected, as adsorption of TB (Kd = 5.4-20, Koc = 380-1100; U.S EPA, 1997) and DTB (less polar than the parent) to soil particles would decrease availability to the plants.

A similar rationale may be responsible for the 10-fold higher IC_{50}s obtained in the agar system compared to the hydroponic system. Both TB and DTB may be stabilized by agar, reducing their availability to rice. A more likely reason for the comparatively low IC_{50} values calculated from this study may be that all the previous studies used initial nominal concentrations of a single dose of TB and DTB, from which IC_{50}s were derived (Tatsuyama *et al.*, 1981; Chen, 2002). Here solutions were renewed every 2 d and the concentrations used to calculate the endpoints were an average of the analytical measurements made over the 48-h exposure renewal periods.

Table 2. Calculated NOEC and IC_{50} values.

	Test	NOEC (μM)	SD	IC_{50} (μM)	SD
Thiobencarb	1	0.61	0.27	2.1	1.4
	2	0.95	0.27	2.0	2.4
	3	0.31	0.076	3.5	2.8
	Means	**0.57**	**0.24**	**2.7**	**1.8**
Deschlorothiobencarb	1	0.070	0.027	0.15	0.21
	2	0.073	0.061	0.32	0.52
	3	0.11	0.090	0.30	0.15
	Means	**0.073***	**0.035**	**0.26***	**0.13**

*Significantly different from TB value ($p < 0.01$).

Table 3. Comparison of IC_{50} values derived from different media.

	Hydroponic Solution[1]	Japanese Soil[2]	Louisiana Soil[3]	Agar[3]
Thiobencarb IC_{50} (ppm)	0.69	30	50	6.2
Deschlorothiobencarb IC_{50} (ppm)	0.058	2.0	5	0.3
TB IC_{50} / DTB IC_{50}	12	15	10	21

[1] This investigation
[2] Tatsuyama *et al.,* 1981
[3] Crowley Silt Loam, Chen, 2002

To determine if DTB has the potency to reduce shoot height in a rice field situation, we considered the stoichiometric relationship between TB and DTB. For DTB to cause toxic effects in the field it must be able to do so at lower molar concentrations than TB. This is because it is produced from TB, making it impossible for DTB to exceed the initial TB concentration. In essence, DTB must have a lower NOEC than TB to cause DPS at concentrations produced by typical field applications. This reasoning is based on the assumption that DPS is not caused by toxicity to the parent compound, that the application rates of TB are safe for rice (i.e. equivalent to or below the NOEC). In this investigation, the NOEC for DTB was found to be significantly lower than that for TB. From the 12-fold difference in toxicity of the two compounds, it can be estimated that a nontoxic application of TB could result in toxic levels of DTB if more than 15% of the TB is reduced, again supporting the hypothesis that DTB is the cause of DPS.

Determining the amount of TB converted to DTB in a field is difficult because it varies widely with soil conditions, probably the reason it seems DPS occurs randomly. Rice straw incorporation was found to accelerate DTB formation. It is thought that the reduction of TB is a co-metabolic process and that organic matter in a field serves as a carbon source for the microbes, facilitating conversion to DTB (Moon and Kuwatsuka, 1984 and 1985b). Copper, especially as antifungal agents, has been correlated to DPS prevention, perhaps by reducing declorinating microbes (Groth *et al.*, 1999). Other soil characteristics such as high phosphate content, low iron content, pH of 7, and temperature between 25-30°C were found to promote the DTB formation in a Japanese paddy soil (Moon and Kuwatsuka, 1984, 1985a, and 1985b), in some cases converting well over 75% of TB to DTB (Moon and Kuwatsuka, 1985b).

For an approximation of how these data might relate to the field, test tube concentrations were compared to the application rate of 26.7 lb. Bolero/acre (C & P Press, 2002) dissolved in 10cm of flood water (soil interactions neglected), which equates to 0.0174 μM (4.49 ppm) TB. This estimation is below the minimum concentrations tested for both TB (0.19 μM) and DTB (0.035 μM) and is well below the TB NOEC calculated here (0.57 ± 0.24 μM), suggesting that if applied properly TB should not injure rice. Although this application rate is also below the DTB NOEC (0.073 ± 0.035 μM), it is within two standard deviations, implying toxicity may occur if most of the TB applied is reduced to DTB.

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