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COMPREHENSIVE RESEARCH ON RICE
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PROJECT TITLE: The Environmental Fate of Pesticides Important to Rice Culture

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OBJECTIVES AND EXPERIMENTS CONDUCTED BY LOCATION TO ACCOMPLISH OBJECTIVES:

Objective I. To investigate the processes governing the fate of penoxsulam (Granite), a new herbicide, in California rice fields. The main research goals for Objective I were to describe the soil sorption and volatility of penoxsulam in typical California rice field conditions.

Objective II. To investigate the natural biological factors governing the environmental movement and fate of pesticides in California rice fields. Emphasis for 2004 was on describing the anaerobic degradation of penoxsulam (DE-638) under California rice field conditions.

Objective III. To compare the potential metabolic activation and/or detoxication of clomazone (Cerano 5 MEG) between rice and watergrasses, and to exploit metabolic differences to develop herbicide safeners. Emphasis for 2004 was on research correlating comparative plant toxicity to the metabolic activation of clomazone (to the toxic metabolite 5-ketoclomazone), as well as transformation of clomazone to non-toxic metabolites.

SUMMARY OF 2004 RESEARCH (MAJOR ACCOMPLISHMENTS) BY OBJECTIVE:

OBJECTIVE I

Introduction

Penoxsulam (DE-638; trade name Granite) is a new herbicide designed for post-emergence control of annual grasses, sedges, and broadleaf weeds (Roberts *et al.*, 2003). Its release is scheduled for 2005 (Robinson, 2003). Penoxsulam is a triazolopyrimidine sulfonamide and belongs to an herbicide group called ALS (acetolactate synthase) inhibitors, which are thought to have low toxicity to mammals, birds, fish, amphibians, or invertebrates and at the same time suppress or stunt weeds at extremely low doses. However, some ALS inhibitors have long

residual field activities field and can damage non-target plants at concentrations so low they cannot be detected by standard analytical procedures (Whitcomb, 1999). Due to its novelty, little information is available on the environmental fate and effects of penoxsulam.

To predict the fate of a pesticide in rice field conditions, information is needed on both partitioning (physical distribution between air, water, and soil) and degradation (breakdown) processes. Pesticide partitioning is controlled by soil sorption (soil-water partitioning) and volatilization (air-water partitioning). The main degradation processes are biodegradation by microbes and photodegradation by sunlight. This continuing study examines the role of all these processes for the fate of penoxsulam in California rice fields. The project consists of controlled laboratory experiments simulating California rice field conditions. The experiments yield the parameters required for a general assessment of the fate and persistence of penoxsulam.

Soil-water partitioning of penoxsulam was studied by the batch equilibrium method, which is the standard procedure to characterize the soil sorption of chemicals (e.g., see OECD, 2000; Wauchope *et al.*, 2002). This method uses soil-water slurries to yield sorption values that can be used to predict soil-water partitioning under a variety of relevant environmental conditions. To this end, soil sorption values for penoxsulam were determined as a function of soil characteristics (e.g., organic carbon content and pH). Sorption values allow general predictions about soil sorption of a chemical in the field. Four representative California rice field soils were used: Sacramento clay, San Joaquin loam, Stockton clay adobe, and Willows clay.

As a general rule, rice pesticides have a greater tendency to volatilize in the Central Valley, with its hot dry summers, than in growing regions with cooler climates. In this study, we assessed the potential for volatilization of penoxsulam by determining its Henry's law constant (K_H). K_H is the *air-water partitioning* ratio at equilibrium:

$$K_H = \frac{c_a}{c_w} \text{ (dimensionless)} \quad (1)$$

where c_a is the concentration of the pesticide in air as moles per liter of air and c_w is the concentration of the pesticide in water as moles per liter of water. K_H can either be estimated based on a chemical's vapor pressure (P^0, Pa) and water solubility (S; mol · L^{-1}):

$$K_H = \frac{P^0}{S}\left(\text{Pa} \cdot \text{L} \cdot \text{mol}^{-1}\right), \quad (2)$$

or it can be experimentally determined. We used the gas-purge method (Mackay *et al.*, 1979), which is the method of choice for experimental determinations of K_H (e.g., Ten Hulscher *et al.*, 1998; Dunnivant *et al.*, 1988).

Methods

Soil-water partitioning by batch equilibration. The soil sorption potential of penoxsulam was evaluated on the four soils most commonly used for rice cultivation in the Sacramento Valley: Sacramento Clay, San Joaquin Loam, Stockton Clay Adobe, and Willows Clay. Soil samples

were collected from the top 20 cm layer of representative locations on April 27, 2004 (San Joaquin Loam) and on May 7, 2004 (Sacramento clay, Stockton clay adobe, Willows clay). Sampling sites were GIS-logged on August 6, 2004. Soils were oven-dried (100°C), disaggregated with a hammer, and homogenized using a mortar and pestle. Larger pieces of plant material were removed with forceps and the soils were sieved to a particle size $\leq$ 1.4 mm. The processed soil samples were kept at 5°C in the departmental cold storage room. The soils were characterized by the DANR Analytical Laboratory at UCD.

In brief, the experimental procedure was as follows. Soil slurries were prepared consisting of radiolabeled penoxsulam solutions at known concentrations in 0.01 M $CaCl_2$ and soil samples of known dry weight. Radiolabeled solutions of penoxsulam were prepared by adding ^{14}C-penoxsulam (0.03 mg L^{-1} ~ 4000 cpm mL^{-1}) to solutions of unlabeled penoxsulam (0/0.07/0.5/1.2/5 mg L^{-1}) in 0.01 M $CaCl_2$. All experimental solutions contained 200 mg L^{-1} $Hg(II)Cl_2$ as a biocide to inhibit biological degradation. To prevent light exposure and photodegradation, all experimental trials were conducted in screw-capped 8-mL amber vials (or 50-mL Nalgene Oak Ridge Teflon FEP tubes wrapped in aluminum foil). Before starting a trial, soils were pre-equilibrated in 0.01 M $CaCl_2$. Soil/solution ratios were determined experimentally for each soil and ranged from 1/1 (Willows Clay) to 1/50 (Stockton Clay Adobe), with the goal being to attain experimental soil/solution ratios, where the percentage of adsorbed penoxsulam was above 20% and below 80% (Wauchope et al., 2002). After addition of penoxsulam, the soil/buffer slurries were agitated for an appropriate time to achieve equilibrium. All soils were at or near equilibrium after 10 h, as determined in a preliminary experiment. After agitation for a sufficient period to achieve equilibrium, the slurries were separated by centrifugation (5 min at 2,500 x g) and a 1 mL aliquot of the aqueous phase was withdrawn and transferred to an 8-mL liquid scintillation vial containing 7 mL of liquid scintillation (LSC) cocktail. ^{14}C-penoxsulam concentrations were determined with a TRI-CARB Liquid Scintillation Analyzer Model 2000CA (Packard; Downers Grove, IL). Based on the measured radiotracer concentration, the soil adsorption was determined by calculating the difference between the amount of test substance initially present in solution (determined by measuring controls) and the amount remaining at the end of the experiment. Controls consisted of solutions of 12 mg L^{-1} unlabeled penoxsulam and 0.03 mg L^{-1} ^{14}C-penoxsulam in 0.01 M $CaCl_2$ and 200 mg L^{-1} $Hg(II)Cl_2$ (no soil). The controls were subjected to precisely the same steps as the test systems. There was no evidence for a loss of penoxsulam by adsorption to the tubes or chemical degradation.

The experimental results were fitted to the Freundlich equation:

$$c_s = K_F \cdot c_w^{\,n} \qquad (3)$$

where c_s is the concentration of penoxsulam adsorbed in soil, c_w is the concentration of penoxsulam in solution, K_f is the Freundlich adsorption coefficient, and n is a constant indicating the nonlinearity of the adsorption isotherm. The soil-water partition constant K_d, the organic matter-water partition constant K_{om}, and the organic carbon-normalized soil-water partition constant K_{oc} where derived from equation 3 by using equations 4-6:

$$K_d = K_F \cdot c_w^{\,n-1} \qquad (4)$$

$$K_{om} = \frac{K_d}{f_{om}} \quad (5)$$

$$K_{oc} = \frac{K_d}{f_{oc}} \quad (6)$$

Air-water partitioning by gas-purge method and model calculations. The Henry's law constant (K_H) describes the relative escaping tendency of a compound existing as vapor molecules as opposed to being dissolved in water (Schwarzenbach *et al.*, 1993). K_H can be measured in an experiment or calculated based on vapor pressure and water solubility data.

Equation 2 was used to calculate K_H at different pHs. Roberts *et al.* (2003) report pH-dependent water solubilities for penoxsulam of 5.7 mg L^{-1} at pH 5, 410 mg L^{-1} at pH 7, and 1,460 mg L^{-1} at pH 9; and a vapor pressure of 9.5 x 10^{-14} Pa at 25°C.

Whenever feasible, a measured value for K_H is preferred, for example, to better account for the temperature dependency of K_H (Ten Hulscher *et al.*, 1998). Thus, we attempted to measure K_H experimentally with the gas-purge method (Mackay *et al.*, 1979), which is one of the methods of choice for the determination of K_H. In this method, an inert gas (e.g. ultrapure nitrogen) is bubbled at a known flow rate through a solution with the chemical in question, and the decrease in the solution phase concentration is monitored over time. K_H can be determined according to

$$K_H = -\frac{kVRT}{G} \quad (7)$$

where k is the first-order dissipation rate constant (s^{-1}), V is the solution volume (m^3), R is the gas constant (8.2058 x 10^{-1} atm · m^3 · K^{-1} · mol^{-1}), T is the temperature, and G is the nitrogen flow rate (m^3 · s^{-1}).

The custom-made gas-purge apparatus consisted of two jacketed, temperature-controlled Pyrex® cylinders containing 1L of a 12 mg L^{-1} penoxsulam solution in 0.01 M $CaCl_2$ and 200 mg L^{-1} $Hg(II)Cl_2$. Glass tube inserts tipped with coarsely fritted disks near the bottom of the vessel bubbled a constant nitrogen stream through the solution for 48 h at a flow rate of 1000 mL min^{-1}. The experiment was conducted in duplicate at 20°C and at 40°C. At certain time intervals, 1-mL duplicate samples of the aqueous solution were withdrawn and transferred to amber 2-mL autosampler vials. Penoxsulam concentrations were measured by liquid chromatography tandem mass spectroscopy (LC/MSMS, see below).

Biodegradation studies (in progress). Transformation rates and pathways of microbial degradation are evaluated using Willows Clay and Stockton Clay Adobe. These trials are conducted using aerobic water/soil system microcosms in PUF-plugged 60 mL Qorpak™ amber wide-mouth bottles (All-Pak Inc., Bridgeville, PA). The microcosms are designed to simulate flooded field conditions with an aerobic water column over an aerobic layer of field soil that is underlain with an anaerobic gradient (OECD, 2002a).

Soil sampling sites have been GIS-logged on August 6, 2004. From each location, soil samples were collected from the top 20 cm layer of flooded rice fields on August 6, 2004, by two methods that allow oxygen-free collection of submerged soils: 1) about 1 kg of water-logged soil was collected in 1-gal Ziplock bags. Before the soil was added, remaining air was completely released from the bags by submerging and opening them in the rice field water above the soil. 2) At each location, ten oxygen-free soil samples were collected in 50-mL Nalgene tubes (Corning Inc., Corning, NY). To avoid exposure of the anaerobic soils to oxygen, the tubes were filled completely with water and then pushed upside-down into the sediment at a vertical angle of ca. 45°. To release water from the bottom of the sampling tube, a Teflon tube of approximately 1 m in length was inserted (for ease of usage, the Teflon tube has a nick ca. 15 cm off the end that is inserted into the Nalgene sampling tube). The soils samples are kept water logged under exclusion of oxygen and light at 5°C.

Before adding XDE-638, a period of acclimation is allowed for the experimental soil/water systems. The period of acclimation is the time needed to reach reasonable stability of the system, as reflected by pH, oxygen concentration in water, and redox potential (OECD, 2002a). In a preliminary trial, the length of the period of acclimation was determined as one week.

Dow AgroSciences has developed a method for extraction and analysis to determine concentrations of penoxsulam and its metabolites in water and soil samples by LC/MS/MS (Roberts *et al.*, 2003). Dow has granted permission to use the existing proprietary method and to adapt it for use in this study; method adaptation is in progress. The extraction procedure in brief: soil samples are transferred to 50-mL Nalgene Oak Ridge Teflon FEP tubes and cloransulam-methyl is added as a surrogate standard to monitor extraction procedure recoveries of the analytes. Subsequently, an extraction solution of 90:10 acetonitrile/1.0 N HCl is added and the centrifuge tubes are capped and shaken for 2 h on a reciprocating shaker at 180 rpm. An aliquot of the extract is evaporated, reconstituted in 0.1 N HCl, and purified by solid phase extraction (SPE). One-mL water samples are directly applied to HLB extraction cartridges for SPE, after adding surrogate standard. For the SPE, the extract is drawn through 30-mg hydrophilic-lipophilic balanced (HLB) extraction cartridges (Waters Corporation, Milford, MA), mounted on a vacuum manifold. The eluate is evaporated and reconstituted in HPLC mobile phase containing 5 ng mL^{-1} stable isotope internal standard ([C^{13}-Ph-UL]-DE-638). Initial recoveries for penoxsulam were 81 $\pm$ 18 % (n = 2) for water samples and 114 $\pm$ 14 % (n = 4) for soil samples.

Chemical analysis was performed with an Agilent Model 1100 LC system (Palo Alto, CA) and an API 2000™ MS/MS (MDS Sciex, South San Francisco, CA). The column was a Luna 3µ C-8 (Phenomenex, Torrence, CA). The mobile phase was 50:50:0.01 acetonitrile:methanol:acetic acid (phase A) and 100:0.01 water:acetic acid (phase B). For elution of penoxsulam, the following elution gradient was used at a flow rate of 150 µL min^{-1} with a total run time of 20 min: start (0 min, 30:70 phase A:B); step 1 (1 min, 100:0); step 2 (8 min, 100:0); step 3 (8.1 min, 30:70); step 4 (20 min, 30:70). Analysis for penoxsulam was performed in positive ionization mode with an electro spray interface. The injection temperature was 450°C. The Q1/Q3

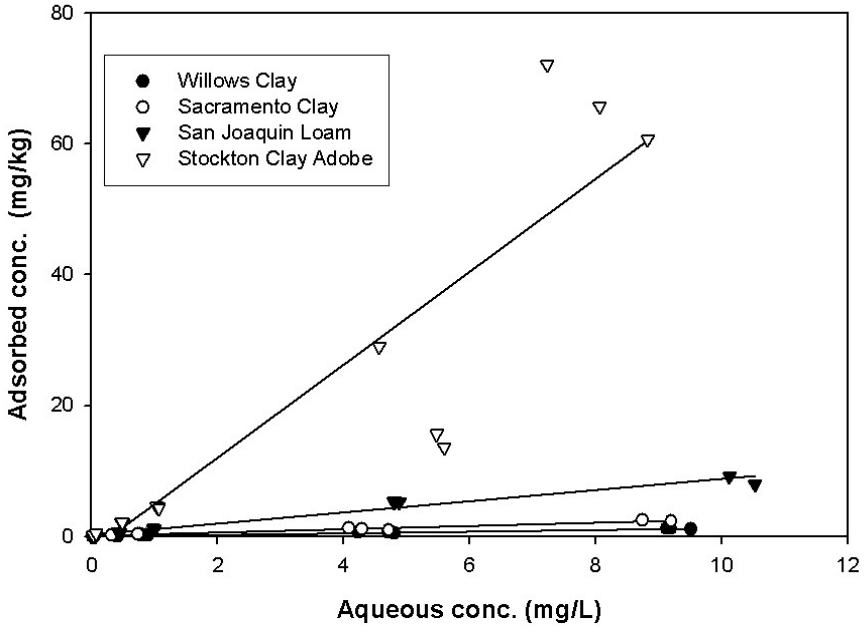


Figure 1. Adsorption of penoxsulam on representative rice field soils of the Sacramento Valley.

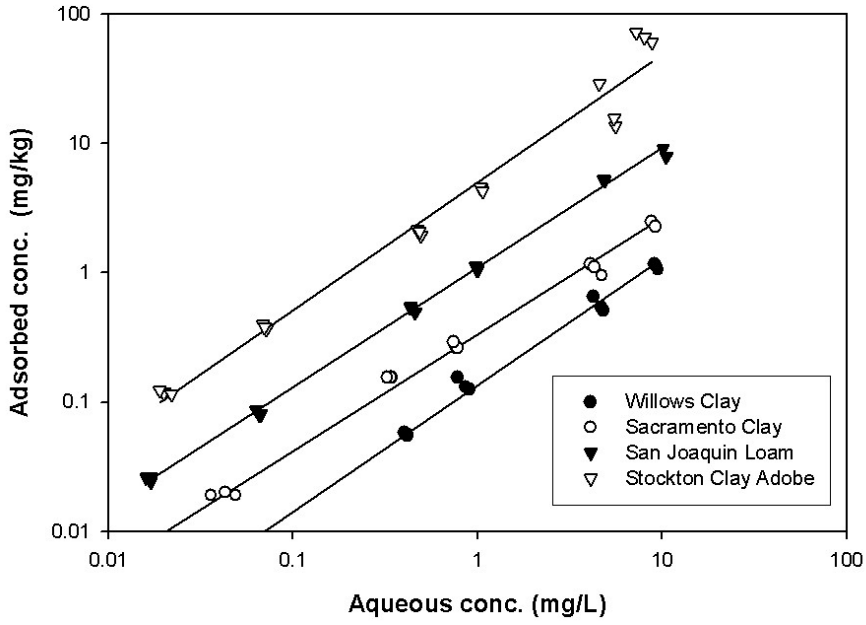


Figure 2. Adsorption of penoxsulam on representative rice field soils of the Sacramento Valley (log-log scale).

ions for penoxsulam, (^{13}C-)penoxsulam, and cloransulam-methyl were 484/193, 485/195, and 430/230, respectively. A separation and detection method for nine degradation products of penoxsulam (provided by Dow) is currently being developed.

Results

Soil-water partitioning. Soil adsorption isotherms of penoxsulam in four representative rice field soils of the Sacramento Valley are shown in Figures 1 and 2. In all cases *n* was close to 1 (Table 1), indicating that the Freundlich adsorption isotherms (see Equation 3) could also be approximated with linear isotherms. This implies that penoxsulam concentrations up to an initial aqueous concentration of 12 mg L^{-1} did not saturate potential sorption sites of the soils. The recommended application rate of 40 g ai/ha would yield field water concentrations well below this value (Roberts, 2003). Hence, the soil-water partitioning of penoxsulam can be considered independent of its initial concentration at environmentally relevant concentrations. Equation 4 can be simplified to:

$$K_d \sim K_F \qquad (8)$$

K_F and the soil-water partitioning constant K_d can be assumed equal for most practical purposes. K_F values for Willows clay, Sacramento clay, San Joaquin loam, and Stockton clay adobe were 0.13, 0.33, 1.10, and 5.00, respectively. There was no significant relationship between the organic carbon and organic matter contents and K_F values. Instead, there was a strong correlation between the soil pH and K_F or K_d (see Table 1 and Figure 3). As Figure 3 indicates, soil sorption increases as the pH decreases. K_F values < 1 imply weak sorption and high mobility of a chemical in soil (OECD, 2000). Hence, penoxsulam can be considered qualitatively mobile in the neutral Willows clay and Sacramento clay soils. In comparison, the mobility of penoxsulam is slightly decreased in Stockton clay adobe and San Joaquin loam, which are acidic soils with pH values of 4.6 and 5.3. Desorption experiments to determine the reversibility of soil sorption in Stockton clay adobe and San Joaquin loam are in progress. In addition, no-expense add-on studies are planned to illuminate further the role of pH and organic matter in the adsorption of penoxsulam to soil.

Air-water partitioning by gas-purge method and model calculations. Figure 4 shows the result of the gas purging experiment, which was an effort to empirically determine K_H of penoxsulam and to examine the effect of temperature on its air-water partitioning. As seen in Figure 4, no changes in the aqueous concentration of penoxsulam were observed in any of the experiments at the maximum feasible flow rate of 1000 mL min^{-1}. It was not possible to obtain an experimental value for K_H. However, the experiment indicates that volatilization from water to air is a negligible pathway for penoxsulam. This is confirmed by the calculated value of K_H. Using Equation 2, K_H was calculated as 1.1 x 10^{-12} Pa · L · mol^{-1}. Chemicals with a K_H lesser than 3 x 10^{-5} Pa · L · mol^{-1} are considered non-volatile and will remain in the water after application.

After turning off the gas and termination of the air/water partitioning experiment, the penoxsulam solution, which included $Hg(II)Cl_2$ as a biocide, was kept in the light-tight and air-tight gas purging vessel and maintained at a temperature of 40°C. Periodically, samples were taken over a period of three weeks. There was no significant decrease in the aqueous

Table 1. Soil sorption parameters of penoxsulam and properties of test soils.

		Freundlich coefficients								
soil	collection site	K_F	n	r^2	K_d[a]	pH	% OC	K_{oc}	% OM	K_{oc}
Sacramento Clay	Schiedel Ranch 39°18'41N, 122°10'41W	0.33	0.90	1.00	0.39	6.5	1.33	30	2.25	17
San Joaquin Loam	Matthews Ranch 39°13'09N, 121°32'46W	1.10	0.93	1.00	1.21	5.3	0.50	242	0.86	141
Stockton Clay Adobe	Thompson Ranch 39°31'22N, 121°55'46W	5.00	0.99	0.98	5.05	4.6	0.74	682	1.28	395
Willows Clay	Maxwell-Dennis Ranch 39°18'41N, 122°10'41W	0.13	0.98	1.00	0.14	6.8	1.12	12	1.95	7

[a] K_d values were calculated by using equation 4 with the datasets used for the Freundlich isotherms.

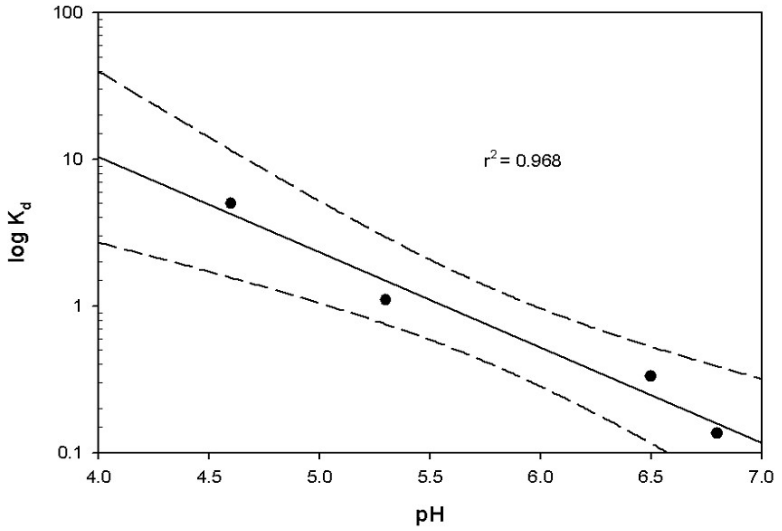


Figure 3. Effect of the soil pH on the Freundlich adsorption coefficient of penoxsulam.

concentration over time (see Figure 5). The lack of degradation indicates the chemical stability of penoxsulam under exclusion of light or organisms.

OBJECTIVE II

Microbial Degradation of Penoxsulam Under Anaerobic Rice Field Conditions

Biodegradation experiments are *in progress* to obtain information about microbial degradation rates and transformation products of penoxsulam in flooded field soils. The study design follows OECD guidelines (OECD, 2002b), and some of the methods are discussed in brief above. Briefly, the experimental system consists of microcosms in 60-mL wide-mouth amber flasks with a 2.5 cm layer of field soil flooded with a water layer of 1-5 cm field water. The bottles are closed with a foam plug that allows gas exchange between the microcosm and the ambient air.

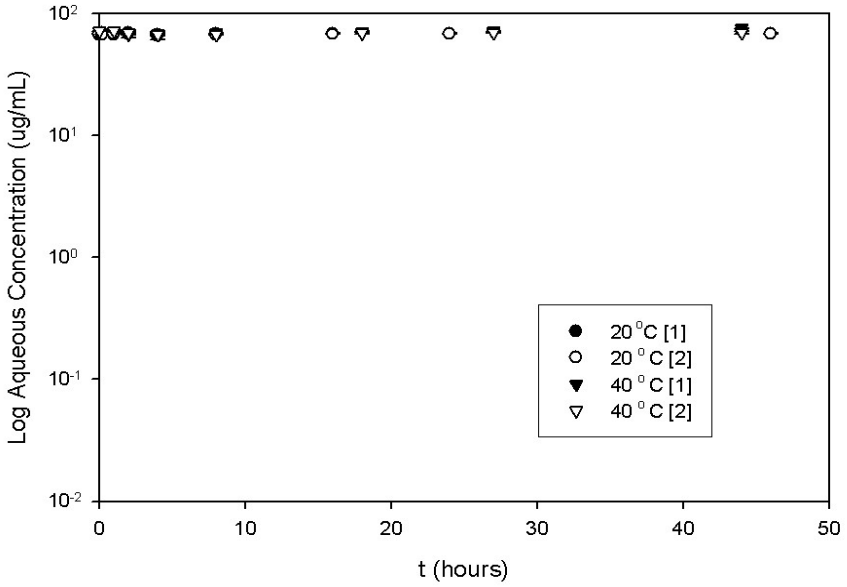


Figure 4. Air-water partitioning of penoxsulam: dissolved concentrations vs. time in the gas-purging experiment.

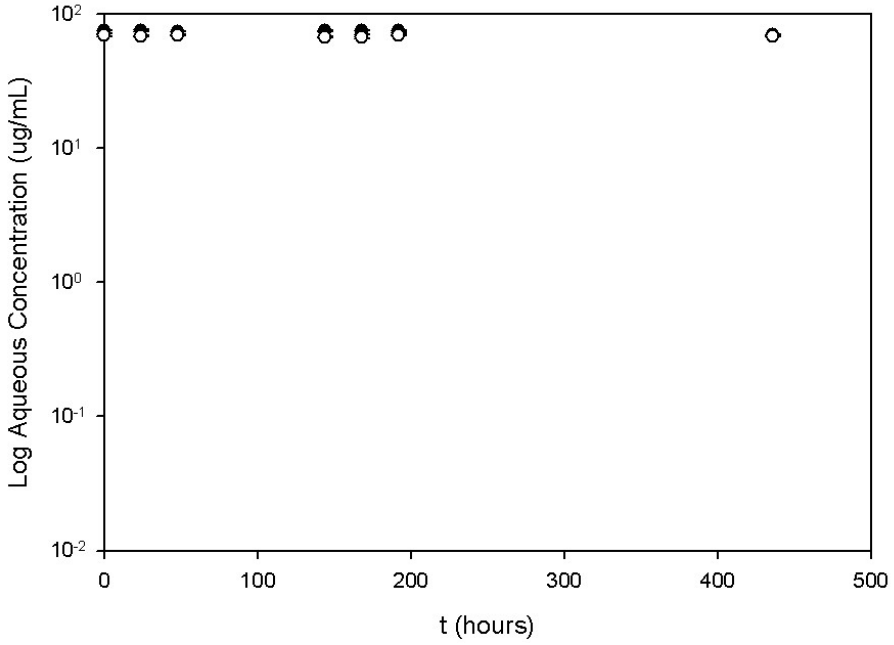


Figure 5. Chemical stability of penoxsulam: dissolved concentrations vs. time in the gas-purging experiment after turning off the gas flow.

This microcosm system simulates an oxygenated water column over an oxygenated sediment layer that is underlain with a gradient from aerobic (oxygenated) to anoxic (oxygen-free) conditions, providing for a realistic simulation of most rice field soil conditions (OECD, 2002a;b). The results will allow estimation of the microbial rate of transformation of penoxsulam and also of the formation and decline of transformation products in the field.

Influence of Copper and Phosphate on Thiobencarb Degradation

Please Note: This study was supported primarily by a Kearney Fellowship to A. Gunasekara.

Introduction

Thiobencarb (*S*-4-chlorobenzyl diethylthiobcarbamate) or Bolero is a common herbicide used in rice agriculture to control annual grasses, such as barnyard grass (*Echinochloa* spp) and certain broadleaf weeds (Reiners *et al.*, 1988). It is a highly effective, non-persistent, systemic pre-emergence herbicide that interferes with protein synthesis and inhibits photosynthesis in weeds (Tomlin, 1994). Thus, it has become a popular rice herbicide that is used in California.

Thiobencarb (TB) is moderately insoluble (30 mg L^{-1}) and most prevalent in the soil (Ishikawa, 1981). The degradation of TB by microbial communities in soil is desirable, however, the dechlorination of the compound in rice fields by microorganisms is problematic because dechlorinated TB, or deschlorothiobencarb (*S*-benzyl diethylthiocarbamate) , has been found to cause dwarfing of rice plants with subsequent loss in rice yields. This phenomenon is known as delayed phytotoxicity syndrome (DPS). Studies by Tatsuyama *et al.* (1981) and Moon and Kuwatsuka (1985) determined that the production of deschlorothiobencarb (DTB) was a microbially-mediated process, but these studies have not examined the soil properties or microbial inhibitors that may contribute to inhibiting the process of TB dechlorination.

This study examined the effect of soil conditions (wet and dried soil) on the degradation of TB and the subsequent formation of DTB, the degradation half-life for TB in two anaerobic rice field soils from the Sacramento valley and the effects of copper (Cu^{+2}) and phosphate (PO_4^{2-}) on the dechlorination and degradation of TB.

Methods

Soils. Soils were collected from two separate rice farm fields in the Sacramento Valley. The farms, Baggett (B) and Mathews (M), are located on the western side of the Sacramento valley and have soils that are characterized as a San Joaquin Series fine mixed thermic Abruptic Durixeralfs. Both farms have experienced DPS. Forty samples from each field were collected in the summer (2003) from each field and homogenized in a conventional blender under a constant nitrogen environment. The homogenized soils were stored at 4°C until used.

Characterization. Soils were characterized for a number of chemical properties by the DANR Analytical Laboratory; analytical methods and results are shown in Table 1. A detailed description of the methods of analysis can be found in Schmelzer *et al.* (2004) or via the DANR website (http://danranlab.ucdavis.edu).

Table 1. Physical-chemical characteristics of soil B and M.

Soil parameter	B	M	Method
Sand (%)	29	32.6	
Silt (%)	45	45.4	Particle size analysis
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	
Nitrogen (%)	0.10	0.13	Carlo Erba Combustion
NH4 –N (mg/kg)	23.1	20.4	
NO3 –N (mg/kg)	<0.1	3.18	
Total Mn (mg/kg)	421.4	547.2	
Total Cu (mg/kg)	67.8	38.2	Microwave digestion followed by
Total Zn (mg/kg)	73.4	66.4	AAS and ICP-AES
Total Fe (g/kg)	28.7	21.9	
Total S (mg/kg)	27.2	32.34	ICP-AES
Exchangeable PO_4-P (mg/kg)	5.55	3.75	Bray
Exchangeable K (mg/kg)	88	72.6	
Exchangeable Na (mg/kg)	27	44.2	Equilibrium extraction followed by
Exchangeable Ca (meq/100g)	7.5	6.3	AES
Exchangeable Mg (meq/100g)	4.4	3.5	

Microcosms. Approximately 6 g wet soil (water content ~ 53%) and 10 mL of untreated well water (Placer County, CA) were placed in clear 60 mL serum bottles which were sealed under a nitrogen environment with butyl stoppers and aluminum crimp caps (Wheaton, Millville, NJ). The serum bottles were placed in a temperature-controlled oven (30°C) in the dark to prevent photolysis of the herbicide. The microcosms were allowed to incubate for 7 d prior to addition of 97 µmol analytical grade TB to any of the treatments; copper hydroxide ($Cu(OH)_2$), copper sulfate ($CuSO_4$), and phosphate (PO_4^{2-}) Low copper (Cu^{2+}) treatments involved adding approximately 12.53 µmol of $CuSO_4$ and 24.83 µmol of $Cu(OH)_2$ to the vials at the same time TB was added. Approximately 0.626 and 1.24 mmol of $CuSO_4$ and $Cu(OH)_2$ was added to the vials as the high copper treatments, respectively, and the PO_4^{2-} amended microcosms (as KH_2PO_4) involved adding 21 mmol of PO_4^{2-} to the vials after the initial 7-d incubation period. All microcosm experiments were run in triplicate (n = 3 at each sampling point) including the controls that had TB in flooded, but autoclaved, soil.

Extraction and organic compound sampling. TB and DTB were extracted using a methanol/hexane solvent extraction procedure. Prior to extraction, approximately 21 µmol of molinate (Syngenta, Richmond, CA) were added to each microcosm. The extraction procedure involved adding 4 mL of methanol to the serum bottle which was vortexed briefly and shaken for 10 min. Then, 3 mL of hexane were added to the bottle, which was vortexed, shaken, and centrifuged at 1000 x g for 10 min. The supernatant was collected and samples were extracted twice more with 4 mL hexane. The supernatant from each hexane extraction was pooled and

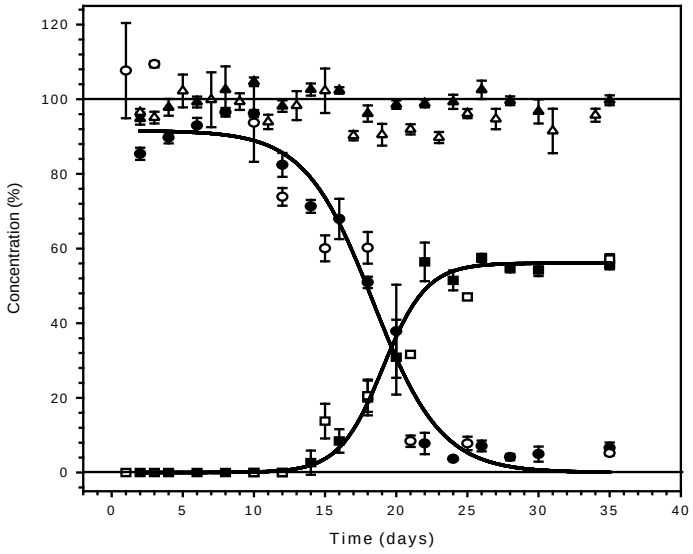


Figure 1. Percent degradation of thiobencarb (97 µmol) using non-sterile dried (?) and permanently wet (?) soil M and the subsequent formation of deschlorothiobencarb (dried = ¦, permanently wet = ?). The sterile wet (?) and dried (?) soils show insignificant loss of thiobencarb over the 35 d sampling period. The solid lines show the logistic degradation and formation for thiobencarb and deschlorothiobencarb in the permanently wet soil, respectively.

concentrated to 5 mL under nitrogen gas. Analysis was conducted via GC-MS using methods established previously in our laboratory for TB and DTB.

Statistics. The $t_{½}$ values reported in this study were calculated using a logistic regression model. Table 2 shows that the logistic model fits the data adequately (r^2>0.841). Microsoft Excel 2002 and Sigmaplot 2000 (version 6.0) graphing software were used to fit the data to the logistic model. Statistical analysis software (SAS Version 8) was used to determine if the slopes of the logistic fit curves were statistically similar or different.

Results and Discussion.

Thiobencarb dechlorination in soils. Fig. 1 shows the degradation of TB and subsequent formation of DTB in flooded non-sterile and sterile microcosms for soil M. The degradation of TB did not occur in the sterile soils, while in the non-sterile soils degradation did occur with subsequent formation of DTB. Fig. 1 also shows that continuously wet soil (anaerobic) had TB degradation and DTB formation patterns similar to the soils that had been dried, during storage (aerobic), before use in the flooded microcosms (Table 2). The data indicate that although the soil was dried, a 7-d incubation time was sufficient for the dry soils to become anoxic in flooded conditions and provide a suitable environment for the revitalization of microbes capable of dechlorinating TB. Similar TB degradation trends were observed for B soils.

Thiobencarb half-life. An important parameter that can be determined from the microbial degradation of TB over time is the $t_{½}$ of the compound. The logistic fit $t_{½}$ results for TB in the non-sterile and low concentration copper amendments are presented in Table 2. The calculated TB $t_{½}$ values for the copper-treated M soils were within ±2 d (12.76-16.83 d) of the untreated

Table 2. Thiobencarb degradation with time and calculated half-life ($t_{½}$) values for thiobencarb in M and B soils.

Soil	Treatment	Thiobencarb remaining (%)				$t_{½}$	r^2
		Day 10	Day 15	Day 21	Day 30	(d)	
M	NS*	94	60	8	5	14.31	0.841
M (dry)	NS*	96	68	38	5	16.83	0.852
M	$CuSO_4$ at 12.53 µmol	71	42	8	-	12.76	0.987
M	$Cu(OH)_2$ at 24.83 µmol	74	68	11	-	14.16	0.882
B	NS*	67	10	10	5	10.88	0.848
B	CuSO4 at 12.53 µmol	31	11	4	-	11.39	0.947
B	$Cu(OH)_2$ at 24.83 µmol	26	12	8	-	10.20	0.911

* Non-sterile

non-sterile wet M soil (14.31 d). Comparisons among the M soils showed that the shortest $t_{½}$ was observed for microcosms having $CuSO_4$ as a treatment at a concentration of 12.53 µmol. The $t_{½}$s for TB in B soils were shorter, in general, when compared to the M soils although the two soils do not vary greatly in physical-chemical properties (Table 1). The $t_{½}$ values for the copper amended B soils were similar to the non-sterile untreated soils. In both soils, the majority of TB was degraded within 30 d. **Please note the low copper concentrations used in this study were five times more than the field-applied rate (4 fl oz a.i. Cu/100 lbs seed). It is expected that application of copper to rice fields at these low rates will not inhibit the formation of DTB.**

Effect of high copper and phosphate concentrations on the dechlorination of thiobencarb. Since the low concentration copper treatments did not inhibit the microbes responsible for the dechlorination of TB, we examined the effect of high copper concentrations on this process. Fig. 2 and 3 show the degradation of TB in sterile M and B soils treated with high copper concentrations. The addition of high levels of copper (0.626 and 1.24 mmol of $CuSO_4$ and $Cu(OH)_2$, respectively) to the M and B soil microcosms revealed contrasting results to that observed for the low concentration treatment. The loss of TB in the microcosms with high copper concentrations closely followed the degradation pattern of the sterile controls in which no DTB formation was observed. At 30 d, there was no DTB found in the non-sterile high copper concentration microcosms.

The effects of PO_4^{2-} (21 mmol) on the degradation of TB over 22 d are presented in Fig. 2 and 3. The PO_4^{2-}-amended microcosms had TB loss of 22 and 37% in M and B soils, respectively. However, no DTB was formed within 22 days which was sufficient time for the dechlorinated chemical species to become observable (Fig. 1). **Please note the phosphorus used in this study (as phosphate) corresponds to and application rate of 19 lbs/acre phosphorus or approximately 43 lbs/acre P_2O_5. At this rate, it is expected that TB dechlorination, and subsequent formation of the toxic deschlorothiobencarb, will be inhibited.**

Statistical analyses (Table 3) of the logistic degradation curves showed that the low concentration copper-treated microcosms were not significantly different from ($P>0.05$) non-sterile untreated microcosm for M and B soils. The high copper and PO_4^{2-} concentrations were

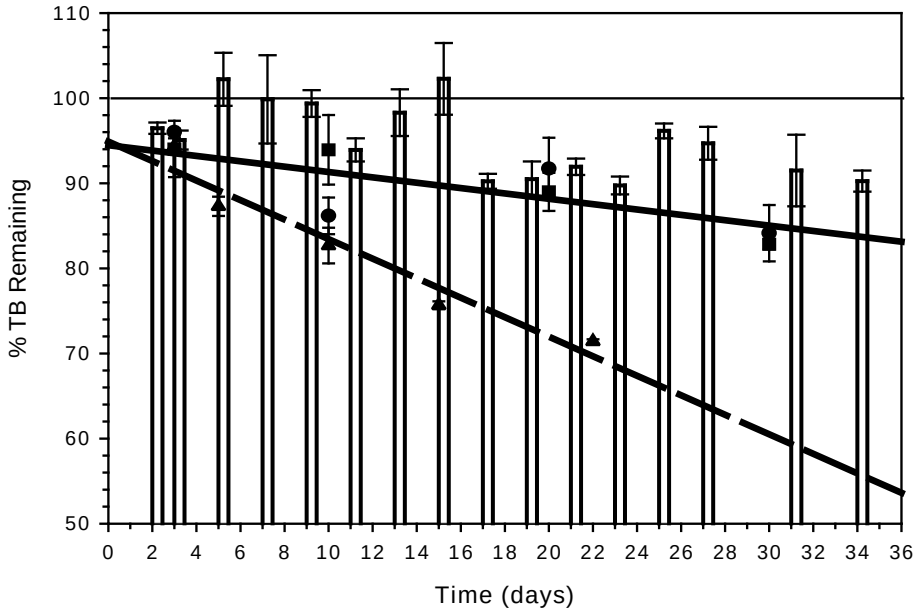


Figure 2. The degradation of thiobencarb in M sterilized soils (vertical bars) and non-sterilized soils with 1.24 mmol $Cu(OH)_2$ (?) , 0.626 mmol $CuSO_4$ (¦) and 21 mmol of PO_4^{2-} (?). The solid line is the relative linear regression for both copper treatments ($Cu(OH)_2$ and $CuSO_4$) while the dashed line is the regression of the phosphate treatment.

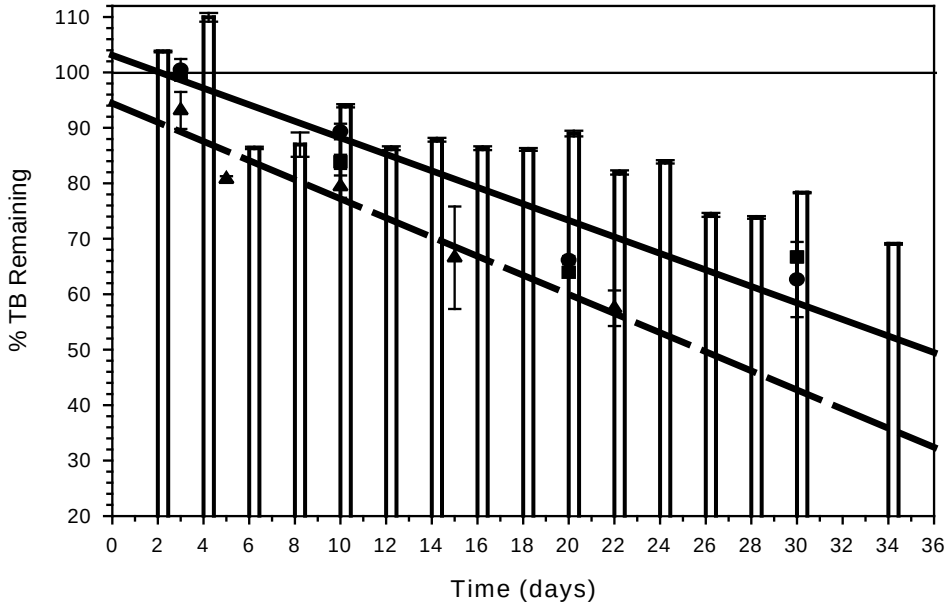


Figure 3. The degradation of thiobencarb in B sterilized soils (vertical bars) and non-sterilized soils with 1.24 mmol $Cu(OH)_2$ (?), 0.626 mmol $CuSO_4$ (¦) and 21 mmol of PO_4 (?).The solid line is the relative linear regression for both copper treatments ($Cu(OH)_2$ and $CuSO_4$) while the dashed line is the regression of the phosphate treatment.

Table 3. Statistical analysis of the logistic decay slopes between the treatments and non-sterile (NS) M and B soils. The significance values (a and b) are groups in which the slopes of the logistic regression are not significantly different.

Soil	Treatment	P value	Significance
M	NS*	-	a
M (dry)	NS*	0.4129	a
M	$CuSO_4$ at 12.53 µmol	0.6983	a
M	$Cu(OH)_2$ at 24.83 µmol	0.6260	a
M	$CuSO_4$ at 0.626 mmol	0.0002	b
M	$Cu(OH)_2$ at 1.24 mmol	0.0002	b
M	PO_4 at 21 mmol	0.0015	b
B	NS*	-	a
B	CuSO4 at 12.53 µmol	0.7801	a
B	$Cu(OH)_2$ at 24.83 µmol	0.9164	a
B	$CuSO_4$ at 0.626 mmol	0.0174	b
B	$Cu(OH)_2$ at 1.24 mmol	0.0261	b
B	PO_4 at 21 mmol	0.0027	b

* Non-sterile

significantly different from the non-sterile untreated B and M soils. Degradation of the low concentration copper and non-sterile M soils did not vary significantly.

The results show that TB dechlorination occurs readily in Sacramento Valley rice soils within 30 d; the process is mainly driven by anaerobic microbes (Fig. 1). The subsequent formation of DTB, which is toxic to rice plants (Palumbo *et al.*, 2004), was also evident in a short time period (within 15 d). Additionally, wet and dry cycles of the soil, present during the growing season and harvest, respectively, did not inhibit the microbes responsible for the dechlorination of TB. This indicates that the microbial populations responsible for the dechlorination of TB in rice field soils are capable of surviving through aerobic dry soil conditions, possibly by spore formation. Spore-forming obligate anaerobes closely resembling the gram-positive Clostridial bacterial group have been identified in rice field soils (Akasaka *et al.*, 2003).

The $t_{½}$ calculations revealed that TB dechlorination occurs in a short period of time under anaerobic rice field soil conditions. These results are in strong contrast to earlier studies that showed TB was very persistent in soils; $t_{½}$ values of >200 d (Walker et al., 1988). The findings may have important implications when considering the role of TB in rice fields because it may be transformed to the dechlorinated compound in a short period of time as observed in this study. For instance, the California Department of Pesticide Regulations require rice water holding times of 30 d to allow for the sorption and dissipation of rice herbicides in the flooded water. This study shows that this time period is adequate when considering the dissipation of TB.

The amendment of 0.626 and 1.24 mmol $CuSO_4$ and $Cu(OH)_2$, respectively, as cupric copper (Cu^{+2}) to the microcosms resulted in no significant different between the dechlorination of TB in test soils vs. sterile soil. Thus, the dechlorination of TB was halted in the presence of high copper additions and indicates that the high copper concentrations inhibited the microbes responsible for

forming DTB. The addition of low copper concentrations (12.53 µmol $CuSO_4$ and 24.83 µmol $Cu(OH)_2$, as Cu^{2+}) to the two soils did not inhibit the dechlorination of TB. This was expected since the background copper concentration was significantly higher than the applied concentration (38 to 68 mg/kg). The high soil background concentration of 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 PO_4^{2-} microcosm results showed a similar trend to that observed in the high copper concentrations with no dechlorination produced over 21 d. However, TB degradation did occur in the presence of PO_4^{2-} over 30 d; 55 and 40% TB degradation for B and M soils respectively. No DTB was found in the microcosms with PO_4^{2-} even though a considerable amount of TB was degraded. The results in this study are consistent with previous work that showed that microbial activity was positively correlated with PO_4^{2-} concentration (Moon and Kuwatsuka, 1985).

In conclusion, this study has shown that the dechlorination of TB in California rice field soils is a microbially-mediated process. The microbes present are able to survive wet/dry cycles and are resistant to low copper concentrations as well as capable of adapting to high background elemental concentrations (copper). The dechlorination can be controlled using high copper or nutrient concentrations. Slight variations in the soil physical-chemical properties do not seem to affect the microbial populations responsible for the dechlorination of TB.

OBJECTIVE III.

Introduction

Studies of metabolism, comparative toxicity, and potential safeners of clomazone (active ingredient in Command and Cerrano) in rice and early watergrass are nearly completed. These two plants showed the largest difference in sensitivity to clomazone in a prior study (TenBrook and Tjeerdema, 2004). Sensitivity differences are likely due to differential activation of clomazone to the toxic 5-ketoclomazone and subsequent detoxification. A study by Culpepper *et al.* (2001) showed that disulfoton and phorate, known P450 inhibitors, protected cotton from clomazone toxicity as measured by growth.

Our studies were designed to determine differences in uptake and detoxification of clomazone in rice and watergrasses, and to explore the effects of pesticides on the toxic action of clomazone. Differential uptake and detoxification were studied by exposing plants to ^{14}C-labeled clomazone. Metabolites were extracted and tentatively identified using HPLC in combination with liquid scintillation counting (LSC). To study the effects of herbicide/pesticide combinations plants were exposed to clomazone alone (and) in combination with disulfoton (active ingredient of DiSyston), piperonyl butoxide (PBO), and demetonS. Disulfoton and PBO are known P450 inhibitors, while demetonS is a P450-activated metabolite of disulfoton which is an esterase inhibitor. Toxic effects were measured by fresh weight and β-carotene levels.

Methods

Study 1. Rice and early watergrass were hydroponically exposed, in closed systems, to ^{14}C-labelled clomazone at 0.05 mg/L for 7 d. Radioactive residues in solution as well as volatile

residues (including CO_2) were collected and quantified to provide a mass balance for clomazone in the system.

Non-polar metabolites were extracted with hexane, while polar metabolites were extracted with 90:10 acetone:water. Extracts were analyzed by HPLC in combination with LSC. Two different HPLC column/solvent systems were used to confirm the identity of eluted compounds based on retention times of analytical standards.

One of the most common detoxification pathways in plants is glucose conjugation. To determine the extent of this reaction, polar extracts were further treated with β-D-glucosidase to remove glucose moieties and then HPLC analyses were repeated.

Study 2. Rice and early watergrass were hydroponically exposed, in closed systems, for 4 d to various concentrations of clomazone in combination with one or more concentrations of disulfoton (0.1 and 0.5 mg L^{-1}), PBO (1.8 and 7.0 mg L^{-1}) and demetonS (0.5 mg L^{-1}). Effects were measured by analysis of fresh weight and β-carotene levels.

Results and Discussion

Study 1.

Table 1 shows that early watergrass absorbed more clomazone, by weight, than rice. This result can provide some explanation for the greater sensitivity of early watergrass to clomazone compared to rice. This is in contrast to studies which found no differential uptake between corn, soybean, smooth pigweed, and velvetleaf (Leibl and Norman, 1991), soybean and velvetleaf (Weimer *at al*., 1992), and tomato and bell pepper (Weston and Barrett,1989).

Table 2 shows the mass balance for clomazone. The percentage of applied clomazone absorbed is very small, likely due to the closed exposure system. Clomazone is very water soluble and hence absorbed via the apoplast system. In a closed system, the vapor pressure in the headspace reduces apoplastic transport. For both species, more clomazone was taken up than was volatilized or mineralized.

Table 1. Differential absorption of clomazone in rice and early watergrass.

	Uptake total (nmol)		Uptake by weight (nmol/g)	
Replicate	Rice	EWG	Rice	EWG
1	8.87	4.02	1.98	3.41
2	6.47	3.99	1.73	3.39
3	8.00	6.13	1.81	5.47
4	7.36	4.99	1.65	4.44
Mean	7.68	4.78	1.79	4.18
se	0.51	0.51	0.07	0.50
p	0.02		0.02	

Table 2. Mass balance for ^{14}C-labeled clomazone.

Rice	uCi	se	n	% total ^{14}C
Solution	2.19	0.03	3	86.1
Volatilized	0.005	0.00	4	0.2
CO_2	0.04	0.01	4	1.5
Plant (extracted)	0.14	0.01	4	5.4
Unextracted/lost in extraction	0.17			6.8
Total	2.54	0.03	4	**100.00**

EWGS	uCi	se	n	%
Solution	2.41	0.04	4	92.5
Volatilized	0.006	0.001	4	0.2
CO_2	0.05	0.003	4	1.9
Plant (extracted)	0.09	0.009	4	3.3
Unextracted/lost in extraction	0.05			2.1
Total	2.61	0.01	4	**100.00**

Fig. 1 compares metabolism of clomazone in rice and watergrasses. Compounds with retention times (RTs) matching those of analytical standards are identified but have not been confirmed. Early watergrass metabolized the absorbed clomazone more extensively than did rice. This level of metabolism can also help explain differential sensitivity since clomazone has to be activated to the toxic 5-ketoclomazone. The P450 enzymes which are likely responsible for this activation are also important detoxifying oxidation reactions.

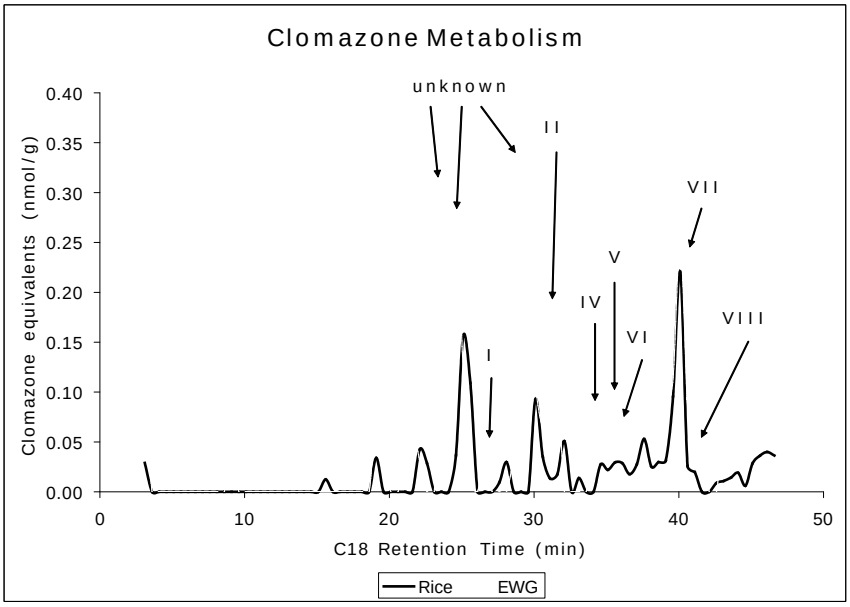


Figure 1. Chromatogram of clomazone and metabolites in rice and early watergrass (EWG). I: 4',5-dihydroxy clomozone; II: 2-chlorobenzoic acid; III: Ring-open reductive product; IV: 3'-hydroxy clomozone; V: 5-hydroxy clomozone; VI: 5'-hydroxy clomozone; VII: clomazone; VII: 5-ketoclomazone.

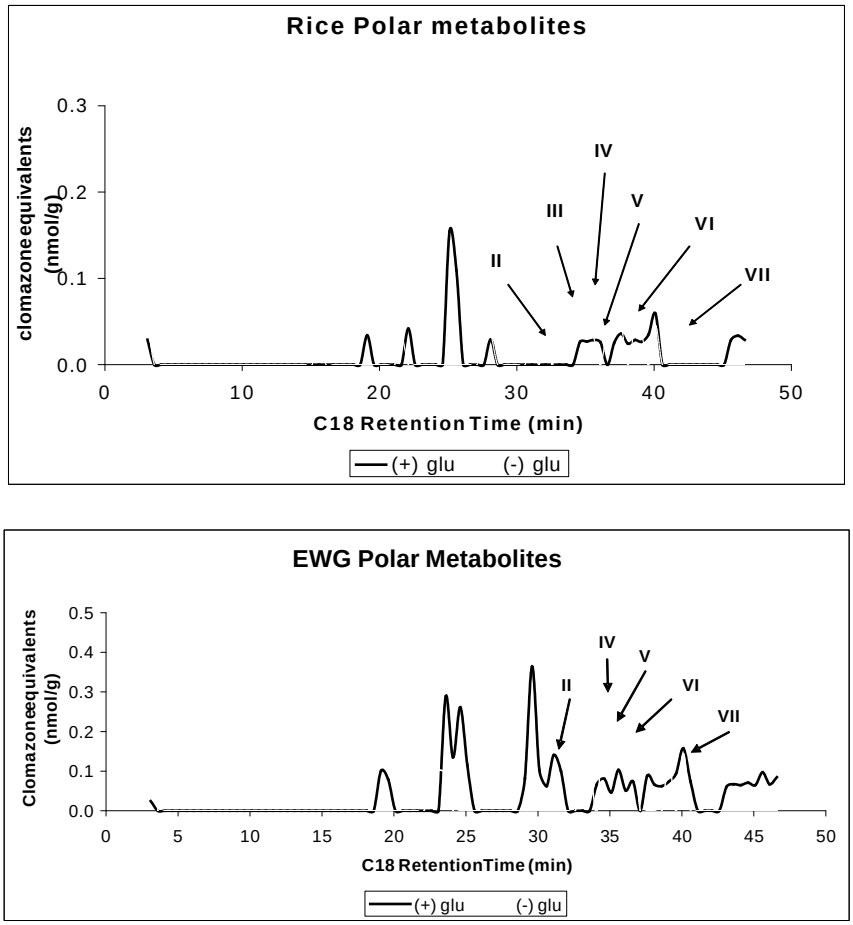
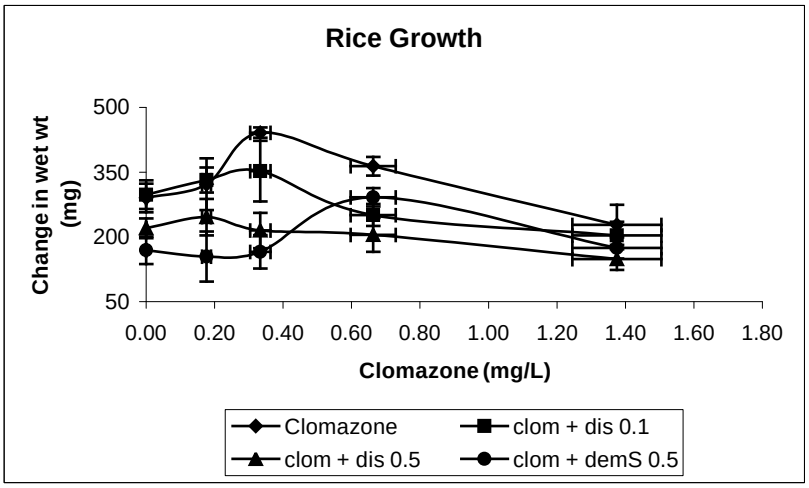


Figure 2. Chromatograms showing polar clomazone metabolites before (+ glu) and after (-glu) treatment with β-D-glucosidase in rice (top) and early watergrass (bottom). I: 4',5-dihydroxy clomozone; II: 2-chlorobenzoic acid; III: Ring-open reductive product; IV: 3'-hydroxy clomozone; V: 5-hydroxy clomozone; VI: 5'-hydroxy clomozone; VII: clomazone; VII: 5-ketoclomazone.

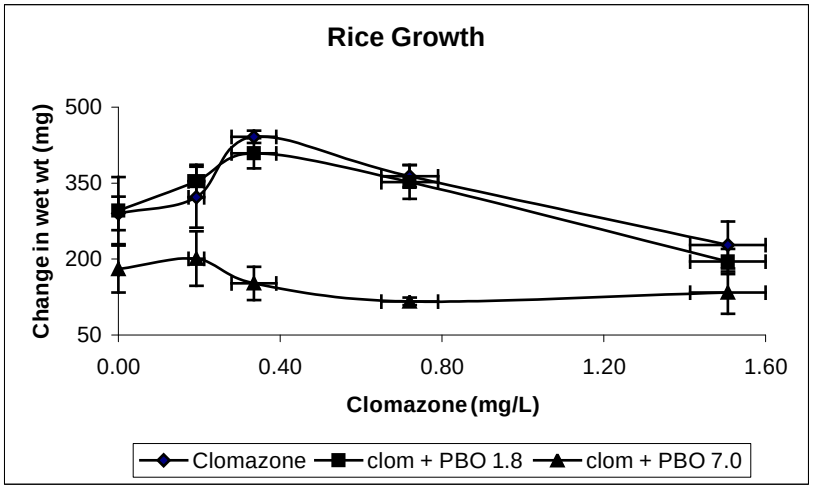
Many unknown compounds were found, particularly in early watergrass. To try to identify these compounds, polar extracts were treated with β-D-glucosidase to cleave glucose-conjugated metabolites. Results are shown in Fig. 2. For both species, peaks that were present before glucosidase treatment disappeared, while new peaks appeared. For both species, the largest new peaks eluted with the hydroxy clomazone standards, indicating that clomazone had undergone a two-step detoxification of hydroxylation, followed by glycosylation. Further confirmatory work is planned with a different HPLC method that uses an HPLC column.

Study 2

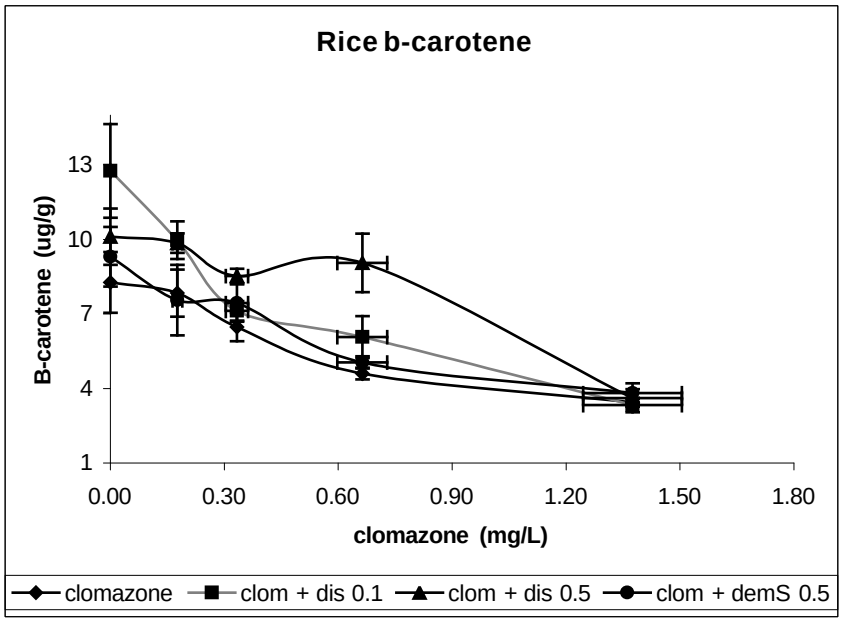
Figure 3 shows dose-response curves for rice and early watergrass exposed to clomazone alone and in combination with disulfoton, PBO or demetonS. Statistical significance of these results has not yet been determined, but some comments can be made. As was expected, clomazone did not affect rice growth at any concentration and had a stimulatory effect below 0.3 mg L^{-1}. PBO at


Rice Growth
Change in wet wt (mg)
500
350
200
50
0.00
0.20
0.40
0.60
0.80
1.00
1.20
1.40
1.60
1.80
Clomazone (mg/L)
Clomazone
clom + dis 0.1
clom + dis 0.5
clom + demS 0.5

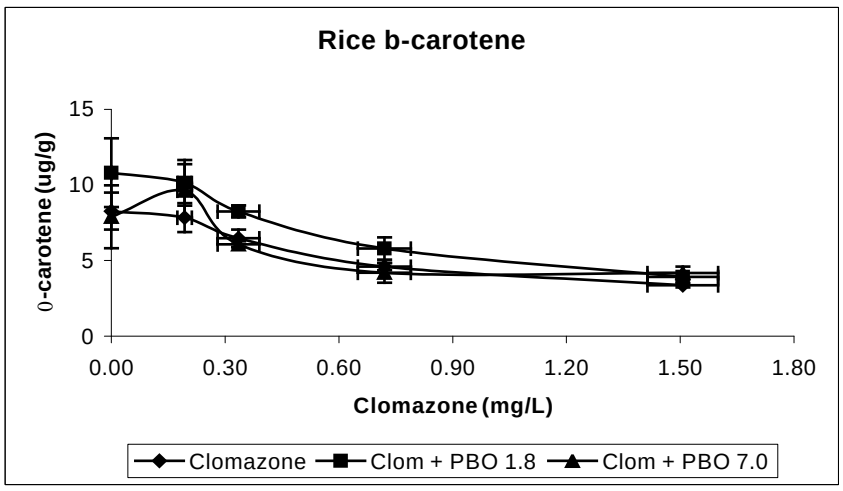
a)


Rice Growth
Change in wet wt (mg)
500
350
200
50
0.00
0.40
0.80
1.20
1.60
Clomazone (mg/L)
Clomazone
clom + PBO 1.8
clom + PBO 7.0

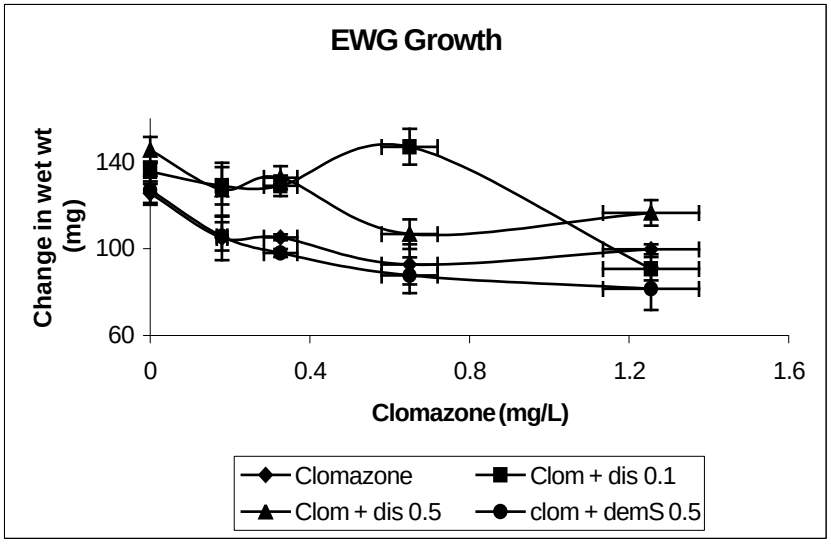
b)


Rice b-carotene
B-carotene (ug/g)
13
10
7
4
1
0.00
0.30
0.60
0.90
1.20
1.50
1.80
clomazone (mg/L)
clomazone
clom + dis 0.1
clom + dis 0.5
clom + demS 0.5

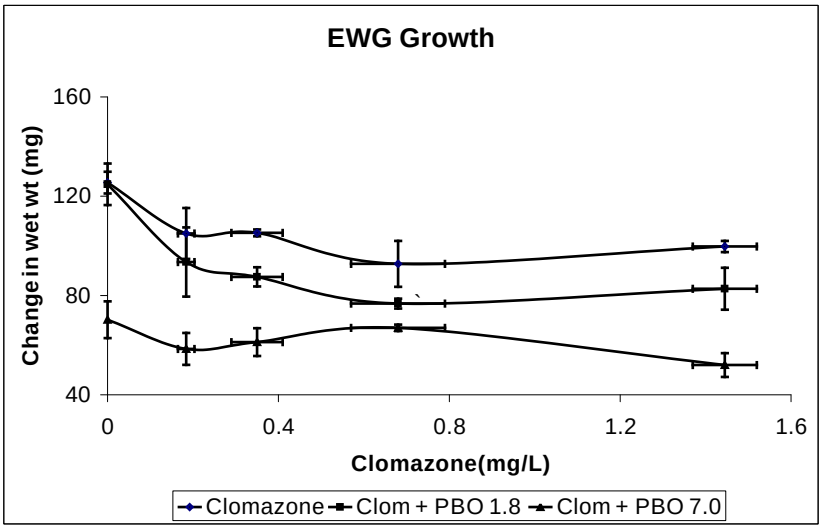
c)


Rice b-carotene
0-carotene (ug/g)
15
10
5
0
0.00
0.30
0.60
0.90
1.20
1.50
1.80
Clomazone (mg/L)
Clomazone
Clom + PBO 1.8
Clom + PBO 7.0

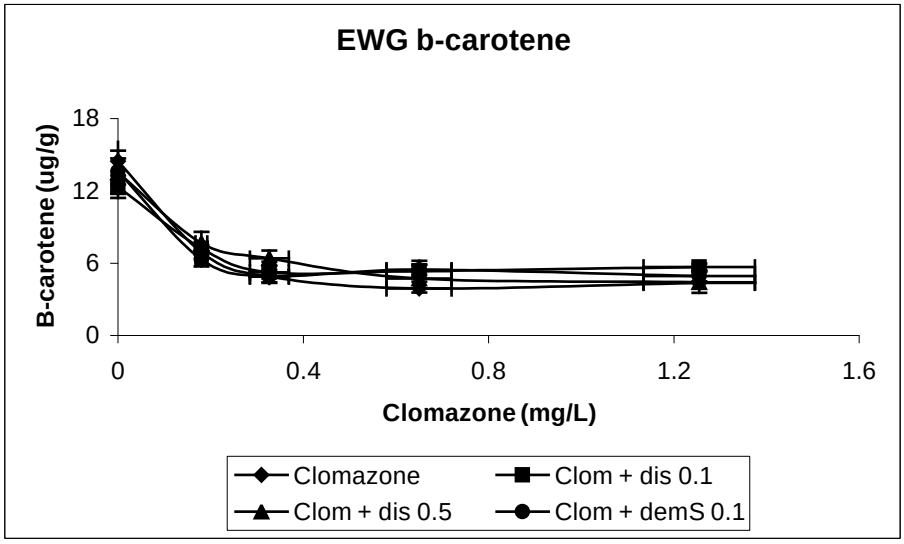
d)


EWG Growth
Change in wet wt (mg)
140
100
60
0
0.4
0.8
1.2
1.6
Clomazone (mg/L)
Clomazone
Clom + dis 0.1
Clom + dis 0.5
clom + demS 0.5

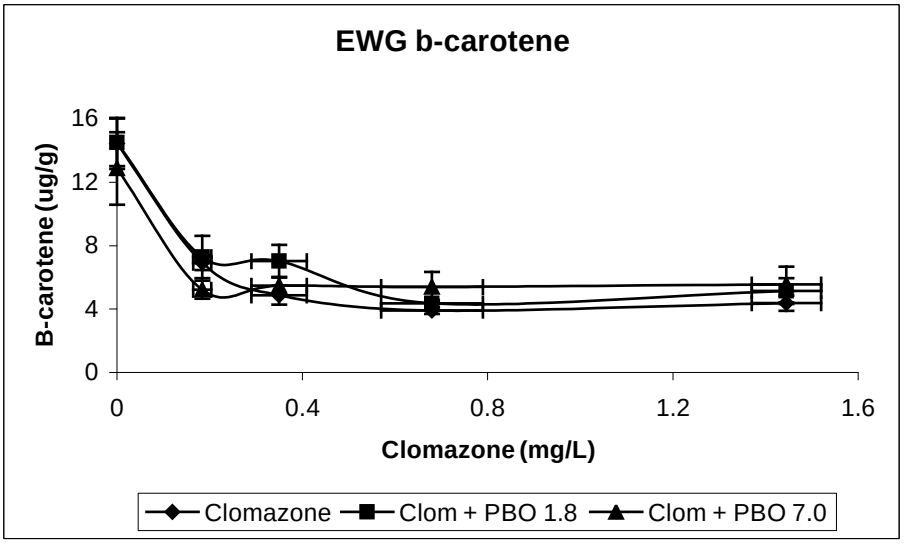
e)


EWG Growth
Change in wet wt (mg)
160
120
80
40
0
0.4
0.8
1.2
1.6
Clomazone(mg/L)
Clomazone
Clom + PBO 1.8
Clom + PBO 7.0

f)



g)



h)

Figure 3. Dose-response curves for rice and early watergrass exposed to clomazone (?), clomazone/disulfoton (?),clomazone/PBO (?)or clomazone/demetonS (°).Effect of different pesticide concentrations and combinations on rice growth (a and b), early watergrass growth (c and d), and β-carotene levels in rice (e and f), and early watergrass (g and h).

7.0 mg L^{-1} had a strong negative effect on growth. With respect to β-carotene levels, 0.1 mg L^{-1} disulfoton had a slight protective effect as did PBO at 1.8 mg L^{-1}. Higher levels of disulfoton and PBO did not alter clomazone's effect on β-carotene levels. DemetonS negatively affected growth, but had no effect on β-carotene in rice.

Disulfoton at 0.1 and 0.5 mg L^{-1} had a protective effect on early watergrass growth, while demetonS had no effect. PBO negatively affected growth of early watergrass at both 1.8 and 7.0 mg L^{-1}. None of the pesticides affected the β-carotene dose-response curves for early watergrass.

Conclusions

These studies were designed to provide some understanding of the mechanisms of differential sensitivity to clomazone which has been observed between rice and early watergrass. Differences have been found between rice and early watergrass in their abilities to absorb and metabolize clomazone, with early watergrass absorbing more and metabolizing it to a greater extent.

Some small safening effects have been observed for clomazone in combination with low levels of disulfoton and PBO. To help understanding the results of this study, the data will be integrated with greenhouse studies of similar herbicide/pesticide combinations.

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CONCISE GENERAL SUMMARY OF CURRENT YEAR'S RESULTS:

1. We used the batch equilibrium method to determine sorption values of penoxsulam in four representative rice field soils from the Sacramento Valley. The soil sorption (K_d) values are smallest in Willows clay (pH = 6.8) and Sacramento clay (pH = 6.5) with mean values of 0.14 and 0.39. K_d values < 1 indicate that penoxsulam is qualitatively mobile and there is no significant retention in soil. In comparison, K_d values were higher in San Joaquin loam (pH = 5.3) and Stockton clay adobe (pH = 4.6), with mean values of 1.16 and 5.00. These indicate that sorption increases slightly as soil pH decreases. **In summary, our soil sorption results indicate that penoxsulam will be highly mobile in water and not significantly retained in rice field soils.**

2. We attempted to measure the Henry's law constant (K_H) of penoxsulam. The K_H is used to evaluate a chemical's propensity to volatilize from field water to air. We employed a gas-purging experiment, which is a state-of-the-art method for measuring K_H. Aqueous solutions of penoxsulam were continuously bubbled with nitrogen over a period of 48 h at 20°C and at 40°C. Even at the higher temperature, vigorous gas purging did not result in any measurable decrease of the penoxsulam concentration in solution. Therefore, it was not possible to determine an experimental value for K_H. **The calculated K_H of penoxsulam is extremely low at 1.1×10^{-12} Pa · L · mol^{-1}. Chemicals with a $K_H < 3 \times 10^{-5}$ Pa · L · mol^{-1} are considered non-volatile.**

3. In summary, the results for Objective I indicate that penoxsulam sorbs only weakly to field soils and has little tendency to volatilize from field water. **Hence, soil sorption and volatilization have little impact on the dissipation of penoxsulam from rice fields. Based on these findings and preliminary results from degradation studies, photodegradation by sunlight and biodegradation by microorganisms are expected to control the dissipation of penoxsulam from Sacramento Valley rice fields.**

4. For Objective II, studies of the microbial degradation of penoxsulam are currently in progress. **With thiobencarb, it was found that phosphate application at currently acceptable field rates could both enhance thiobencarb degradation and inhibit the formation of deschlorothiobencarb, which would inhibit the occurrence of delayed phytotoxicity syndrome.** Copper addition was a less effective or acceptable means for reducing deschlorothiobencarb production.

5. For Objective III, it was found that early watergrass possesses a well-developed capability for metabolizing clomazone to potentially less toxic metabolites. Neither rice nor early watergrass appear to produce measurable amounts of the putative toxic agent, 5-ketoclomazone. **Early watergrass is capable of absorbing clomazone at almost twice the rate as rice, possibly explaining the differential sensitivity towards the watergrass.**

6. Experiments to elucidate the role of safeners in improving the effectiveness of clomazone (in Collaboration with Albert Fischer) are underway and should be completed by the summer of 2005.