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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 factors governing pesticide dissipation in California rice fields. Emphasis for 2013 was to complete characterization of the soil sorption of the insecticide clothianidin (Belay) under California rice field conditions.

Objective II. To investigate the factors governing pesticide dissipation in California rice fields. Emphasis for 2013 was to complete characterization of the influence of hydrolysis on sorption of the herbicide imazosulfuron (League) under California rice field conditions.

Objective III. To investigate the factors governing pesticide dissipation in California rice fields. Emphasis for 2013 was to complete characterization of the volatilization of the herbicide benzobicyclon (Butte) under California rice field conditions.

SUMMARY OF 2013 RESEARCH (MAJOR ACCOMPLISHMENTS) BY OBJECTIVE:

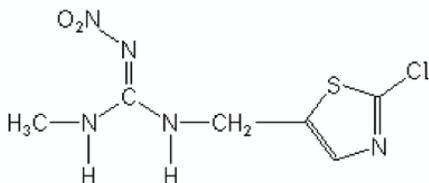**Objective I – Soil-Water Partitioning of Clothianidin (Belay)**

Figure 1: Structure of Belay, a neonicotinoid insecticide.

Introduction

Belay (clothianidin; Figure 1), a synthetic neonicotinoid insecticide, acts as a neurotoxin in a wide variety of insects including Hemiptera, Thysanoptera, Coleoptera, Lepidoptera and Diptera (Jeschke *et al.,* 2003; Tomizawa and Casida, 2005; Umene 2006). Belay use in agricultural areas of California is low; however, it had the largest increase in use in 2011 as a number of products containing it were recently registered for use on cotton, cucumber, grapes and tomatoes (DPR, 2011). It is currently being registered in California for pre- and post-flood aerial application to protect rice fields against the rice seed midge (*Cricotopus sylvestris*) and rice water weevil (*Lissorhoptrus oryzophilus*).

Reported air-water partitioning coefficient (*H*) values for Belay are low, indicating that it will not readily vaporize from water into the atmosphere (USEPA, 2010). However, these values were calculated by measuring the ratio of its vapor pressure to water solubility at 25^oC (no values are reported for elevated temperatures; USEPA, 2010; Umene, 2006; Federoff, 2005). In 2011, we determined that the *H* values for Belay, at temperatures relevant to California rice fields, are low (1.2E-9 and 3.34E-9 Pa m3 mol-1 at 22^oC and 35^oC, respectively). Thus, our results suggest that volatilization will not be a significant route of dissipation for Belay.

Partitioning of Belay between soil and water (K_d) was determined by the batch equilibrium method. Belay sorption affinity, capacity, desorption and organic carbon-normalized (K_{oc}) distribution coefficients were determined using four soils collected from rice fields in the Sacramento Valley. The measured log K_{oc} values ranged between 2.6 to 2.7 at 22^oC and 37^oC. Thus, the soil sorption capacity of rice field soils for Belay is low and decreased at elevated temperatures (37^oC). No significant relationship between soil pH or cation-exchange capacity (CEC) and K_{oc} values was observed.

Materials and Methods

Chemicals. Analytical Belay (99.9% purity) was purchased from Sigma Aldrich (St. Louis, MO, USA). All solvents used were HPLC grade and purchased from Sigma Aldrich. Calcium chloride and ammonium acetate were purchased from Fisher Scientific (Hampton, NH, USA). A stock solution of Belay (100 ug/mL) was prepared and stored in amber bottles at 4^0C. Dilutions were prepared with an aqueous calcium chloride solution (0.01M) to achieve final concentrations.

Soil collection and classification. Soil sorption-desorption potential for Belay was evaluated for four soils taken from rice fields in northern California: Davis, Biggs and from two different fields in Richvale. Several kilograms of each soil were randomly collected from the 0-10 cm layer, air dried and ground with a porcelain mortar and pestle to pass through a 2-mm sieve. In order to inhibit microbial activity, soils were autoclaved at 120^0C for 1 h and then dried at 105^0C for 5 h. Physical-chemical properties were determined by the ANR Analytical Laboratory at UCD and are summarized in Table 1.

Table 1. Selected properties of four rice field soils.

soil	texture	collection site	Post Harvest Straw Disposal	silt (%)	clay (%)	sand (%)	OC (%)	pH	CEC (mequiv/ 100 g)
DfT	silty clay loam	Davis 38:33:22N 121:38:48W	Till	32	44	24	1.29	6.60	37.9
BfB	clay	Biggs 39:27:39N 121:43:57W	Burn	32	48	9	1.59	4.69	20.0
RvB	clay	Richvale 39:29:22N 121:44:12W	Burn	40	24	35	1.97	4.56	5.88
RvT	clay	Richvale 39:29:22N 121:44:12W	Till	36	26	40	1.25	4.46	5.97

Sorption-Water Partitioning. Soil isotherms were constructed using the USEPA batch equilibration method (OECD 106). All isotherms were constructed using triplicate samples and blanks to calculate standard deviation, and sorbed concentration was calculated from mass balance. Isotherms were obtained using 50 mL amber PTFE screw top centrifuge tubes. Calcium chloride was used as a background solution in order to control the ionic strength of the soil solution and to promote flocculation. Preliminary kinetic experiments determined that a soil to solution ratio of 1:2 allowed for 20-85% sorption; apparent sorption equilibrium was reached after 72 h. For each soil experiment, 5.0 g was suspended in 9 mL of 0.01 M $CaCl_2$ and mixed on a rotary shaker (200 rpm) for 12 h before Belay addition. An aliquot of 1 mL stock solution in 0.01M $CaCl_2$ was added to each experiment to achieve initial concentrations of 0.05, 0.1, 0.15, 0.2, 0.5, 0.8, 1, 2, 5, and 10 ug mL^{-1}. Isotherms were run at 22 ± 2^0C for each soil and at 37 ± 2^0C for the Richvale soils.

Suspensions were agitated on a rotary shaker at either 22 ± 2^0C or 37 ± 2^0C at 200 rpm for 72 h. Equilibrium phases were separated by centrifugation (2,000 x g) for 30 min, and 7 mL of supernatant was decanted from each experiment. A 1-mL aliquot of supernatant was passed through a 13-mm Acrodisc syringe tip filter with a 0.45 um PTFE filter (Pall, Port Washington, NY). Controls prepared either without soil or without Belay stock solution were run in triplicate for each set of experiments to show that 1) no interfering compounds were detected in the soil, and 2) Belay was stable and did not sorb to the surface of the centrifuge tube (less than 3.32% loss).

The amount of Belay sorbed to soil after equilibration was calculated as the difference between the initial and equilibrium solution concentration using the equation:

$$q_{eq}^s = \left(C_i - C_{eq}^s\right) * \frac{V_i}{m}$$

where q_{eq}^s is the amount of Belay sorbed by soil at equilibrium (ug/g), C_i and C_{eq}^s are the initial and equilibrium Belay concentrations (ug/mL), V_i is the soil solution volume (mL), and *m* is the mass of soil (g).

Desorption experiments were conducted as a continuation of the sorption isotherm experiments using the conventional single-step decant-refill technique. For each sample, 7 mL of fresh 0.01M $CaCl_2$ was added to replace the discarded supernatant. Samples were placed back onto the rotary shaker and agitated for an additional 72 h and processed as described above. Mass balance calculations established the amount of Belay desorbed
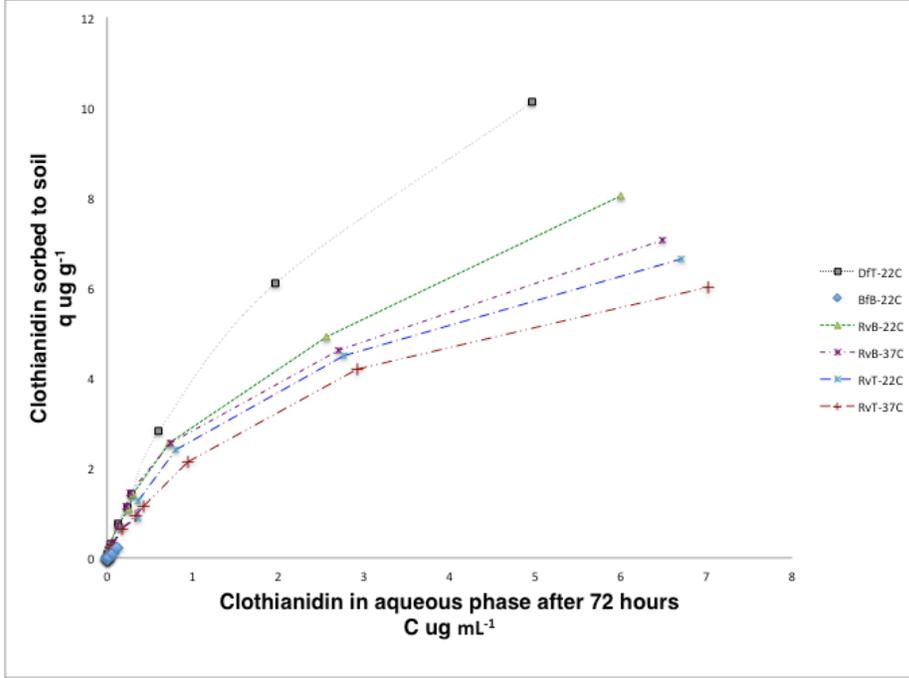
Figure 2. Adsorption isotherms are L-type, thus sorption affinity of Belay decreased as aqueous concentrations were increased for both the 22°C and 37°C treatments.

from the soil.

The amount of Belay desorbed was calculated as the difference between the initial amount sorbed and the aqueous phase concentration of Belay corrected for incomplete decant according to the equation:

$$q_{eq}^d = q_{eq}^s - \left(\frac{c_{eq}^d - \left(\frac{(V_i - V_r)}{V_i} \right)}{m} * V_i \right)$$

where q_{eq}^d is the amount of Belay remaining sorbed by the soil (ug/g), C_{eq}^d and C_{eq}^s are the concentrations in solution at desorption and sorption equilibrium, respectively, V_i and V_r are the initial and replacement solution volumes (mL), and m is the mass of soil (g).

Isotherm linearity was evaluated by fitting all sorption-desorption data to the logarithmic form of the Freundlich equation:

$$\log q_{eq} = N \log C_{eq} + \log K_f$$

where K_f is the Freundlich coefficient, N is the Freundlich exponent, q_{eq} represents q_{eq}^s and q_{eq}^d in the sorption and desorption case, respectively, and C_{eq} represents C_{eq}^s and C_{eq}^d in the sorption and desorption case, respectively.

Table 2. Freundlich sorption and desorption intercept K_F and exponent N and standard error values.

Soil	Temperature	Sorption			Desorption		
		log K_f	N	R^2	log $K_f{}^d$	N^d	R^2
RvT	37 C	0.288 (0.013)	0.708 (0.012)	0.992	1.136 (0.075)	0.916 (0.047)	0.932
RvT	22 C	0.335 (0.020)	0.721 (0.018)	0.982	0.916 (0.070)	0.790 (0.046)	0.917
RvB	37 C	0.425 (0.019)	0.666 (0.016)	0.984	1.452 (0.085)	0.916 (0.047)	0.932
RvB	22 C	0.441 (0.012)	0.681 (0.010)	0.994	0.916 (0.070)	0.790 (0.046)	0.917
BfB	22 C	0.473 (0.017)	0.688 (0.014)	0.989	1.077 (0.044)	0.774 (0.027)	0.967
DfT	22 C	0.547 (0.013)	0.784 (0.012)	0.994	0.614 (0.014)	0.728 (0.010)	0.995

Sorption data were used to calculate soil-water distribution coefficients, K_d, and the experimental organic carbon-normalized sorption coefficients, K_{oc}, for aqueous equilibrium concentrations with initial aqueous loadings of $C_i = 0.1$, 1.0, and 10.0 ug/mL. K_d and K_{oc} values were calculated according to the following equations:

$$K_d = \frac{q_d^s}{c_d^s}$$

$$K_{oc} = \frac{K_d}{f_{oc}}$$

where K_d and K_{oc} have units of L/kg, and f_{oc} is the fraction of organic carbon in the sediment.

Analysis. An Agilent 1100 HPLC with an 1100 series autosampler coupled to PE SCIEX model AP2000 triple quadrapole mass spectrometer equipped with an atmospheric pressure chemical ionization source (APCI) and controlled by Analyst interface software version 1.4.2 was used. It was equipped with a 3.5-µm particle size, 4.6 x 250 mm ID Xterra C18 column (Waters, Milford, MA) using an isocratic mobile phase of 70% methanol with 0.1% formic acid and 30% water with 0.1% formic acid at 400 µL/min flow rate at ambient temperature with a 50 µL injection volume. Detection used MRM in the positive ion mode using the protonated molecular ions $(M-H)^+$ of 250 m/z as the precursor ion. A 7-point external calibration curve was constructed from the peak area of triplicate injections of Belay standards prepared in 0.01M $CaCl_2$. The calibration curve was weighted as 1/x and linear from 0.005 to 10 ug/mL with a regression coefficient of $R^2 = 0.9998$ and residuals less than 10%.

Statistical Analysis. Data were analyzed using JMP software package version 10.0 (SAS Institute Inc., Cary, NC). The Freundlich parameter values K_f and N, standard errors, and coefficient of determination R^2 were obtained from the results of a linear regression analysis of the log-transformed sorption and desorption data, *equation 3*. Standard errors were used to distinguish differences between the Freundlich isotherm parameters K_f and
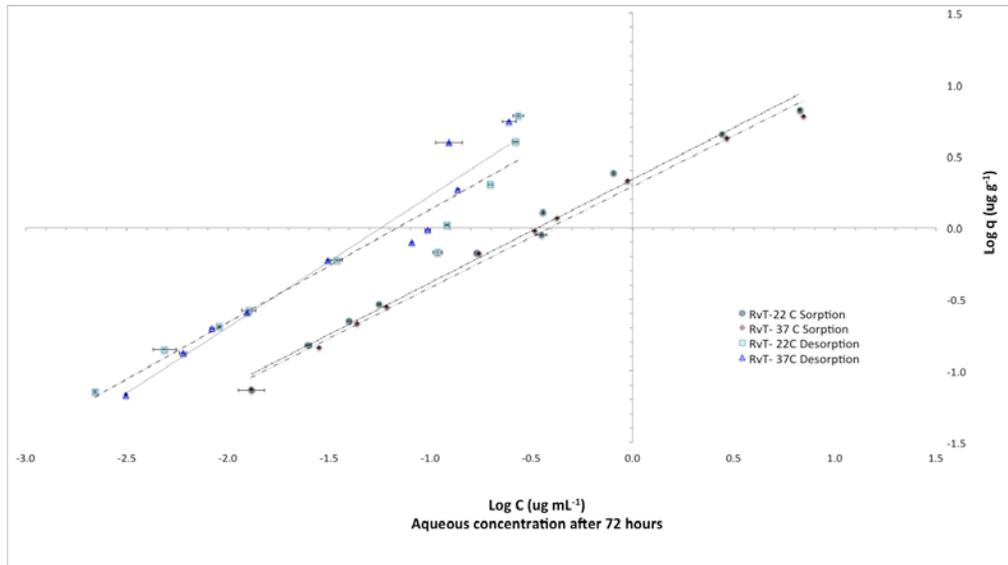
Figure 3. Log-transformed Freundlich sorption and desorption isotherms for RvT soil at 22^0C and 37^0C.

N. Discrete differences among sorption and desorption isotherms (hysteresis), soils, and temperature treatments were determined by calculating the p-values associated with one-way ANOVA test and post hoc comparison (Tukey HSD test, $\alpha = 0.05$). Results with p-values < 0.05 were considered statistically significant.

Results

Soil-Water Partitioning. Isotherms for Belay in four rice field soils representative of the Sacramento Valley are shown in Figure 2. The convex curvature of the sorption data shown in Figure 2 may be described as L-type, indicating that sorption affinity of Belay decreased as aqueous concentrations increased for both 22°C and 37°C temperature treatments (Calvet, 1989). Data for the four rice soils were log-transformed to fit the Freundlich model using Equation 3; corresponding Freundlich parameters K_f and *N*, their standard error values and R^2 values are listed in Table 2.

The log-transformed Freundlich adsorption and desorption isotherms for Belay, on selected rice field soils, are shown in Figure 3 for 22°C and 37°C isotherms. In all cases, sorption isotherms were non-linear (N<1) indicating that soil sorption capacity for Belay decreases as the initial concentration of pesticide increases (Table 2). The Freundlich coefficient K_f is an empirical value, and is a measure of the sorption capacity of a soil for a pesticide; low K_f values indicate decreased sorption capacity and increased leaching potential (Schwarzenbach 2003). Sorption isotherms exhibited low log K_f values ranging between 0.335 to 0.547 and 0.288 to 0.425 at 22°C and 37°C, respectively (Table 2). Furthermore, significant decreases in log K_f values and a greater degree of non-linearity were observed in the 37°C isotherms (p< 0.05). These results indicate that 1) the sorption capacity of rice field soils for clothianidin is low and 2) Belay leaching potential will be dependent upon the amount of Belay applied, and 3) sorption capacity will decrease at

Table 3. Soil-water partitioning coefficient Kd and organic carbon-normalized partitioning coefficient Koc values calculated for Ci of 0.1 ug mL^{-1}.

Soil	f_{oc}	Temperature	Kd (L kg -1) calculated at initial concentration Ci of 0.1 ug mL	log K oc (L kg -1) calculated at initial concentration Ci of 0.1 ug mL
RvT	0.0125	37 C	5.08	2.61
		22 C	6.06	2.69
RvB	0.0197	37 C	9.80	2.70
		22 C	10.26	2.72
BfB	0.159	22 C	10.82	2.83
RvB	0.0197	22 C	7.86	2.78

elevated temperatures.

In order to compare soil sorption capacities between the four rice field soils, K_d and K_{oc} values were calculated for initial an concentration, C_i, of 0.1 µg mL^{-1} using Equations 4 and 5, and presented in Table 3. Log K_{oc} values ranged between 2.6 to 2.7 at 22°C and 37°C (Table 3). No significant relationship between the soil pH or CEC and K_d values was observed. The lack of dependency on pH and CEC is consistent with the fact that Belay (pKa 11.0) is a neutral species within environmentally-relevant pH ranges (USEPA 2004). The log K_{oc} values for Belay on rice field soils are low and sorption compared with more hydrophobic rice pesticides like etofenprox (aqueous solubility of 405 µg L^{-1}), which has log K_{oc} between 6.0 to 6.4 and 6.1 L kg^{-1} at 22 C and 37 C, respectively for similar rice field soils (Vasquez, 2010).

Desorption. Desorption processes affect pesticide mobility and hysteresis; sorption and desorption are important factors in assessing a pesticide's fate and transport (Huang 1997, Boivin 2005). Desorption isotherms describe the amount of Belay remaining sorbed to soil at desorption equilibrium. Data from the isotherm experiments for the four rice soils were log-transformed to fit the Freundlich model using Equation 3 and the corresponding Freundlich parameters K_f^d and N^d, their standard error values, number of observations n, and R^2 values are listed in Table 2. Hysteresis is characterized by significant differences between the slopes of adsorption and desorption isotherms (Hung 1997; Lima 2010; Bronzic 2012). One-way ANOVA tests and post-hoc comparisons (Tukey HSD test, $\alpha = 0.05$) confirmed that hysteresis was observed in all four rice field soils at 22°C and 37°C.

The desorption experiments and observed hysteric behavior demonstrate that sorbed Belay is slightly stable on rice field soils. There was a significant relationship between the initial concentration of Belay and the percentage of desorption observed ($p < 0.05$). For initial loadings of 0.05 ug mL^{-1} the mean percentages of Belay that remained sorbed to soil after 72 h were 93.6 ± 0.01 and 94.1 ± 0.01 for the 22°C and 37°C treatments, respectively. This indicates that even under field conditions (i.e. elevated temperatures of a hot summer day) bound Belay residues will remain bound to soil. Therefore, although
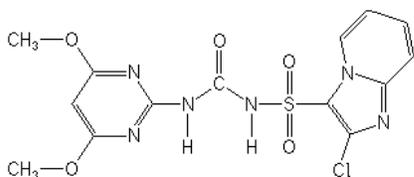
Figure 4. Structure of League.

the sorption capacity of rice field soils is low, dissipation from rice field soils will be dependent upon abiotic and biotic modes of degradation on the soil surface.

Conclusions

Belay is currently being registered for aerial application in California rice fields. In order develop sustainable management practices it is necessary to understand the partitioning behavior between air, water, and soil. Even under elevated temperatures, Belay is nonvolatile and remains primarily in the aqueous phase. The measured log K_{oc} values ranged between 2.6 to 2.7 at 22°C and 37°C in representative rice field soils. These findings suggest that overall sorption capacity of rice field soils is low and that sorption capacity will decrease at elevated temperatures. The data also suggest that sorption to soil is primarily a function of soil organic matter content, and that the properties of the mineral phase are less important. Sorbed residues are not easily desorbed from the studied rice field soils. Overall, Belay has the potential to be mobile in soils though surface degradation may prove to be a fundamental component in the dissipation of Belay from California rice fields. Further investigation should focus on biological and photochemical processes and potential offsite transport in tail water.

Objective II – Sorption of Imazosulfuron (League)

Introduction

Imazosulfuron (League; Figure 4) is a sulfonylurea herbicide of interest to California rice farmers due to its rice selectivity and high effectiveness at a low application rate (75-95 g ha^{-1}; Tomlin, 1997). The USEPA registered League for commercial and residential use in 2010. League's mode of action involves inhibiting the enzyme acetolactate synthase, a critical component to the production of amino acids necessary for new cell generation. League-tolerant plants are thought to rapidly metabolize the herbicide (Tanaka & Yoshikawa, 1994).

Our previous work suggests that once League is applied, it will remain in field water due to its low potential for volatilization or soil sorption. Pesticides that partition into water generally have greater mobility in the environment and a higher potential to contaminate surface and ground water. Understanding the persistence of League in a California rice field will allow for a better estimate of the potential for off-site movement.
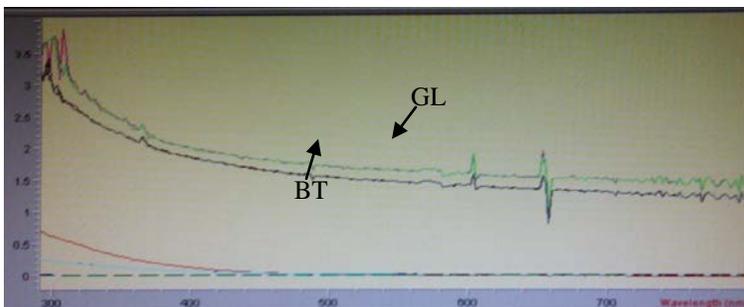
Figure 5. Absorbance of water samples from Glenn (GL) and Butte (BT) counties.

Two dissipation processes important to many pesticides used on rice are currently being investigated: photolysis and hydrolysis. Photolytic reactions are categorized as direct or indirect. Direct photolysis is the process where light is absorbed by a chemical and the energy dissipated by breaking bonds. Indirect photolysis involves a light-absorbing reactive species being generated and subsequently attacking the compound of interest (Crosby, 1998). Because League absorbs light above 290 nm, the cut-off for solar radiation reaching the earth's surface, it has the potential to undergo direct photolysis. Previous studies characterizing League's photodegradation have been limited. Morrica *et al.* (2004) studied League's dissipation in deionized water, and report a half-life of 8 h. Because light intensity is not reported, this data is of limited value. Field water differs significantly from deionized water, and this study neglects to examine influences like turbidity, which may scatter light and increase a chemical's persistence, or natural photoreactive species (photosensitizers), which may promote more rapid degradation. Tagaki *et al.* (2012) examined League's photolysis in field water in Japan, reporting a half-life of 8.5 days. This study suffers from similar issues; radiation time and intensity were not reported, limiting the data's value. Conditions such as temperature, photoreactive species and sunlight intensity differ between Japan and California.

Hydrolysis is believed to be an important dissipation process for League, particularly under acidic conditions. Morrica *et al*. (2001) investigated the hydrolysis of League from pH 1.9 – 12.3 in deionized water under ambient temperatures, finding that it hydrolyzed quickly at low pH ($t_{1/2}$ = 6.3 days, pH 3.6±0.1) and high temperature; it was stable to hydrolysis at neutral or alkaline pHs. However, no studies have examined League's photolysis and hydrolysis under California rice field conditions.

Materials and Methods

Experimental Design. League (1-(2-chloroimidazo[1,2-*a*]pyridin-3-ylsulfonyl)-3-(4,6-dimethoxypyrimidin-2-yl) urea) was purchased from Santa Cruz Biotechnologies, Inc. (Santa Cruz, CA). ADPM (2-amino-4,6-dimethoxypyrimidine), IPSN (2-chloroimidazo [1,2-α] pyridine-3-sulfonamide) and UDPM (2-ureido-2,6-dimethoxypyrimidine) were synthesized, purified and characterized by our collaborator, David Ball (CSU Chico). Standard solutions were prepared in acetonitrile and kept at -20°C, and all solvents were HPLC grade.
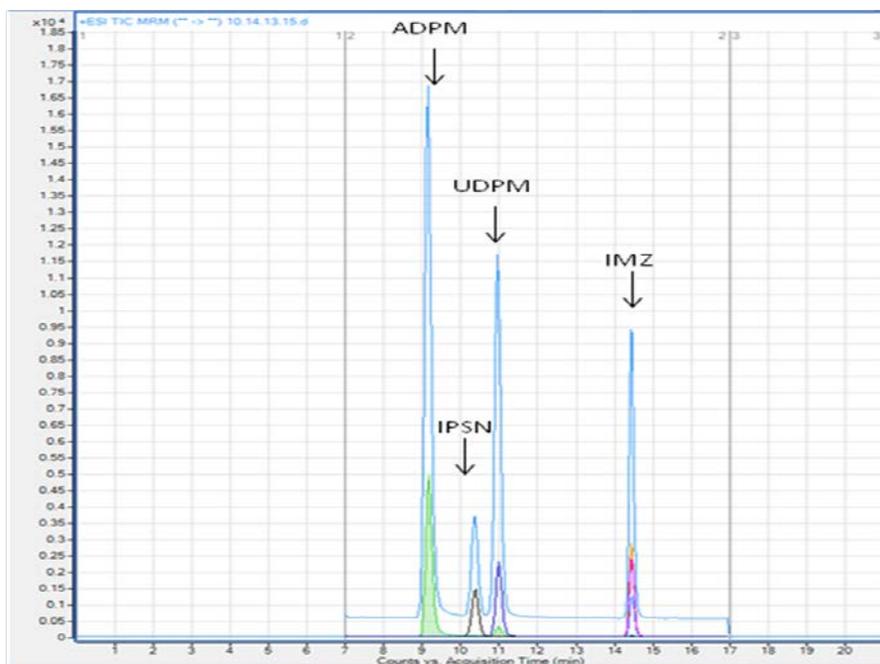
Figure 6: Chromatogram of 50 µg L^{-1} standard.

Field water was collected 2-3 weeks prior to the experiments from Butte (BT) and Glenn (GL) counties in the Central Valley, California and stored at 4°C prior to use. Buffered deionized water (pH 7.0) was used as a control. Water and glassware were autoclaved (121°C, 15 psi) for 30 min prior to use. Each water source was analyzed by a spectrophotometer to check light absorption between 290 – 800 nm (Figure 5).

Sterilized water samples were fortified with League at 200 µg L^{-1} (approximately 4-times field rate) and allowed to equilibrate, stirring for an hour. To each 60-mL borosilicate type-200 vial (filtered out <290 nm; Wheaton Science, Millville, NJ), 20 mL was delivered. Vials were placed in an exposure chamber (34.5°C±1.5), outfitted with eleven (8 W) broad UV (300±50 nm) spectrum lights (Southern New England, Branford, CT). Controls were wrapped in aluminum foil to protect from light. Samples and control were run in triplicate. Irradiation in the chamber was measured using a portable radiometer (7.25 W m^{-2}). Readings taken midday on the roof of Meyer hall in August fell between 7.2-12.9 W m^{-2}. At each time interval, vials were shaken for 10 min at 200 rpm on a shaker and a 3.0 mL volume was removed from each vial, for centrifugation (3500 x *g* for 30 min). The supernatant was frozen at -20°C prior to analysis.

Analysis. LC/MS/MS analysis was performed on an Agilent 6420 liquid chromatograph triple quadrupole mass spectrometer. The column was an Elcipse XDB-C18, 150 x 4.6 mm i.d., 5 µm particle size (Agilent) and was held at 25°C. League and its degradation products were eluted via a mobile phase gradient consisting of 0.25% acetic acid and 0.25% acetic acid in acetonitrile. The flow rate was 0.25 mL/min from the start of the run until 17.0 min then ramped to 1 mL/min to rapidly equilibrate the column. Percent
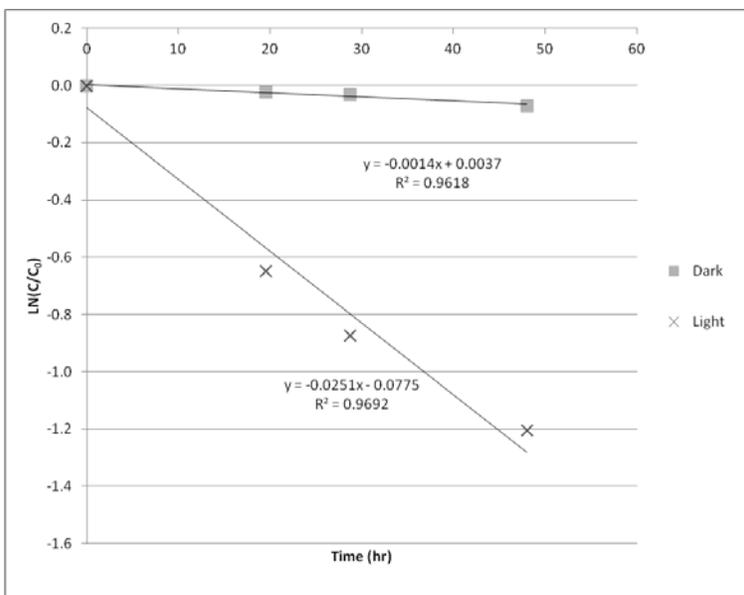
Figure 7. Loss of League in irradiated BT field water and dark controls.

acetonitrile was ramped from the initial condition of 30% to 90% at 0-4 min, then held for 6 min, before ramping down to 30% from 10 - 15 min. Total run time was 21 min, but only eluent from 7-17 min was sent to the MS. MS data were acquired in positive ion ESI mode. A representative chromatogram is shown in Figure 6. Quantification was performed by comparison with a six point calibration curve using the peak areas and a linear regression. Accuracy within ±10% was considered acceptable, except for at the limit of quantification, where ±15% accuracy was required.

The natural log of the imazosulfuron concentration [$\ln(C_t/C_0)$] was calculated and first-order plots constructed. Photolysis half-lives were calculated using the equation:

$$t_{1/2} = \frac{\ln(2)}{k}$$

where k is the absolute value of the slope and the first-order rate constant.

Preliminary Results and Discussion

League dissipation in dark controls was significantly less than loss in irradiated samples, but not negligible (Figure 7). Over the course of the exposure, <10% loss was observed from initial concentrations, which is most likely attributed to hydrolysis. The hydrolysis half-life was calculated to be 495 h (~20 days).

Photolytic half-lives for League were relatively short (Table 4). Statistically significant differences were observed in League's half life between water sources. Faster degradation was observed in Butte County field water than in that from Glenn County (Figure 8). Degradation of League was faster still in deionized water (data not shown).
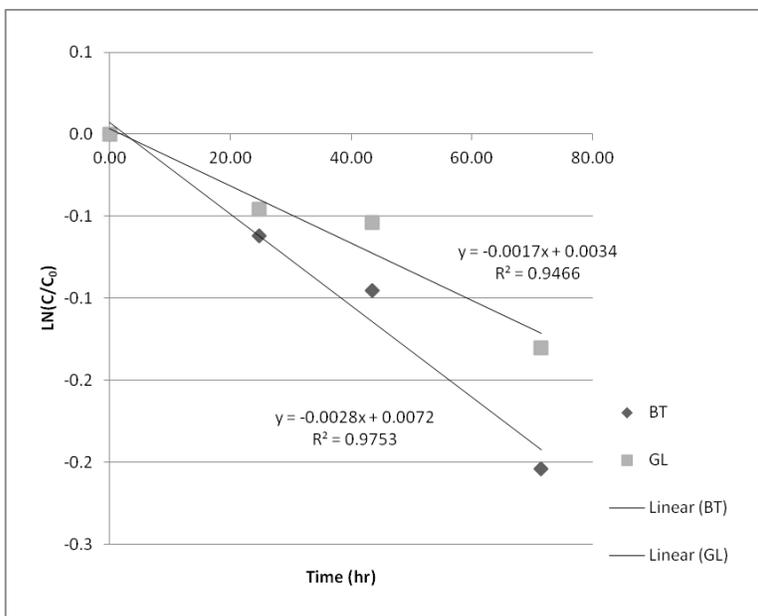
Figure 8. Loss of League in field water from two locations (BT & GL).

Table 4. Summary of first-order reaction rates and half lives of League.

Water Source	First-order Rate Constant (k) (h^{-1})	Half Life (t$_{1/2}$) (h)
Deionized water	0.0421	16.5
Butte county field water	0.0134	51.7
Glenn county field water	0.0087	79.7

These results correlate well with the absorption observed in the water sources. Glenn county field water had higher overall light absorbance and exhibited the longer half life. This suggests that light attenuation is an important factor to League's photolysis rates and that direct photodegradation is an important process to its dissipation in a rice field.

Conclusions

Photolysis is a process that is particularly important in California, where summers are characterized by intense solar radiation. League is a sulfonylurea herbicide which absorbs light >290 nm, giving it the potential to undergo direct photolysis. Preliminary studies suggest that *League rapidly degrades in sunlight* and light attenuation is an important factor in dissipation rates. Previous photolytic studies of League in the literature also suggest photolysis is an important dissipation process. Hydrolysis is a more minor dissipation process, but is not negligible. Future work will more thoroughly investigate both photolysis and hydrolysis of League with fully characterized field water samples.

Objective III. Volatilization of Benzobicyclon (Butte)

Introduction

The synthetic herbicide Butte (benzobicyclon) has been proposed as an effective tool for rice farmers in the Sacramento Valley due to its effectiveness against sulfonylurea-resistant weeds such as *Scirpus juncoides*. Though Butte has not yet been registered for use in California, it is currently used in Japan with great success (Koyanagi and Nakahara, 2009). Butte is considered to be a pro-herbicide; reaction with water (hydrolysis) releases the active herbicide (the hydrolysate) in water, plants and soil (Sekino *et al.*, 1991). Butte hydrolysate (BH) inhibits 4-hydroxyphenylpyruvate dioxygenase (4-HPPD) in weeds, leading to chlorophyll destruction, bleaching and death.

To understand the behavior of benzobicyclon in California rice fields, both chemodynamic transport (movement between phases such as air-water or soil-water partitioning) and degradation (via microbes and/or sunlight) need to be considered. The air-water partitioning behavior of Butte is currently under study, which will aid the prediction of its potential volatility under California rice field conditions. Partitioning depends on both the vapor pressure and the water solubility of the chemical and is measured by Henry's law constant (*H*; Schwarzenbach *et al.*, 2003). Prediction of the volatility of benzobicyclon under California rice field conditions (e.g. 25^oC and 37^oC) can be conducted by determining *H* for Butte.

Calculations

Henry's law constants can be calculated from the vapor pressure and the water solubility of a chemical using the following equation:

$$H = \frac{vapor.pressure}{so\,\text{lub}\,ility}$$

As vapor pressure and water solubility are both dependent on temperature, it is important to calculate *H* values for different temperatures California rice fields might experience. This may be done both experimentally and theoretically (Schwarzenbach *et al.*, 2003).

Methods

The gas stripping apparatus was used to experimentally determine *H* for Butte (Lau *et al.*, 2006). Duplicate columns (1 m x 51 mm inner diameter) were run simultaneously. Approx. 4.1 L of dilute 0.01M $CaCl_2$ solution (pH 7.25) was spiked with 1 mg Butte and equilibrated for 1 h, at which time approx. 1.5 L of this solution was added to each gas-stripping column. High purity N_2 gas was filtered through a hydrocarbon trap with a variable flow rate (40-100 mL/min) and saturated with water before being bubbled through the bottom of each water column. Solid-phase extraction (SPE) cartridges were used to collect volatilized Butte, and a mist trap was used to prevent the exposure of the SPE cartridges to water droplets, which could bias results.
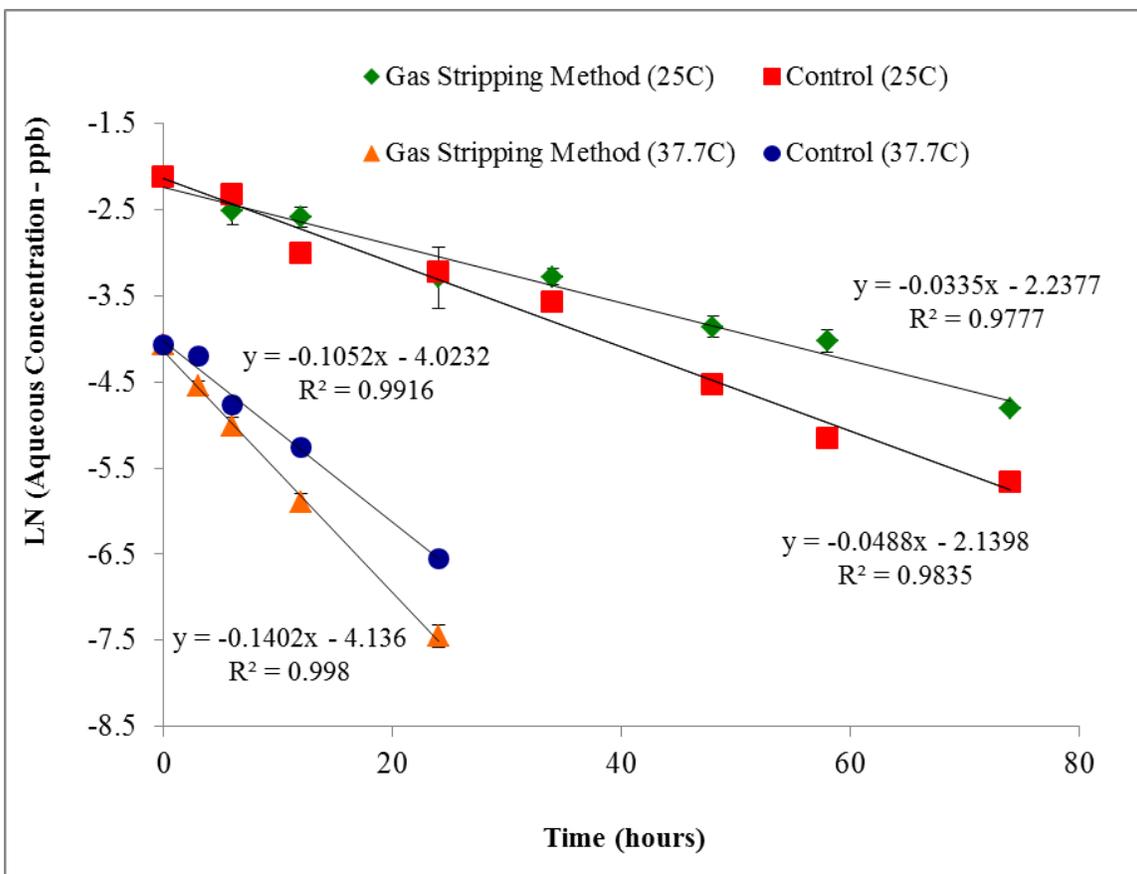
Figure 9: Concentration of aqueous Butte over the course of the experiments (n=2).

Column temperature was maintained using a recirculating water bath, and columns, SPE cartridges and mist traps were foil-wrapped to prevent loss due to photodegradation. Over the course of the experiment, 5 mL water samples were collected to determine the aqueous Butte concentration over time. After experiment completion the Butte solution was drained from the columns, which were rinsed with solvent and analyzed for Butte sorbed to the glass. Acetonitrile was used to elute Butte from the SPE cartridges and dichloromethane was used to elute Butte sorbed to the inside of the apparatus.

A control was also implemented for both the 25°C and 37°C experiments which consisted of a 1-L glass bottle into which 1 L of the same dilute aqueous Butte solution as was used for the gas stripping apparatus was poured. The control was capped and shaken at the same temperature as the respective experiment. Aqueous samples were taken from the control at the same time points as for the experiment. Once the experiment was complete, the bottle was drained and then rinsed with dichloromethane to elute sorbed Butte.

Extraction and Analysis

Water samples were extracted via solid phase extraction (SPE) using Agilent Bond-Elut cartridges previously activated with 5 mL of methanol, followed by a 10-mL rinse of HPLC-grade water. A water sample (5 mL) was then added to the cartridges. After drying
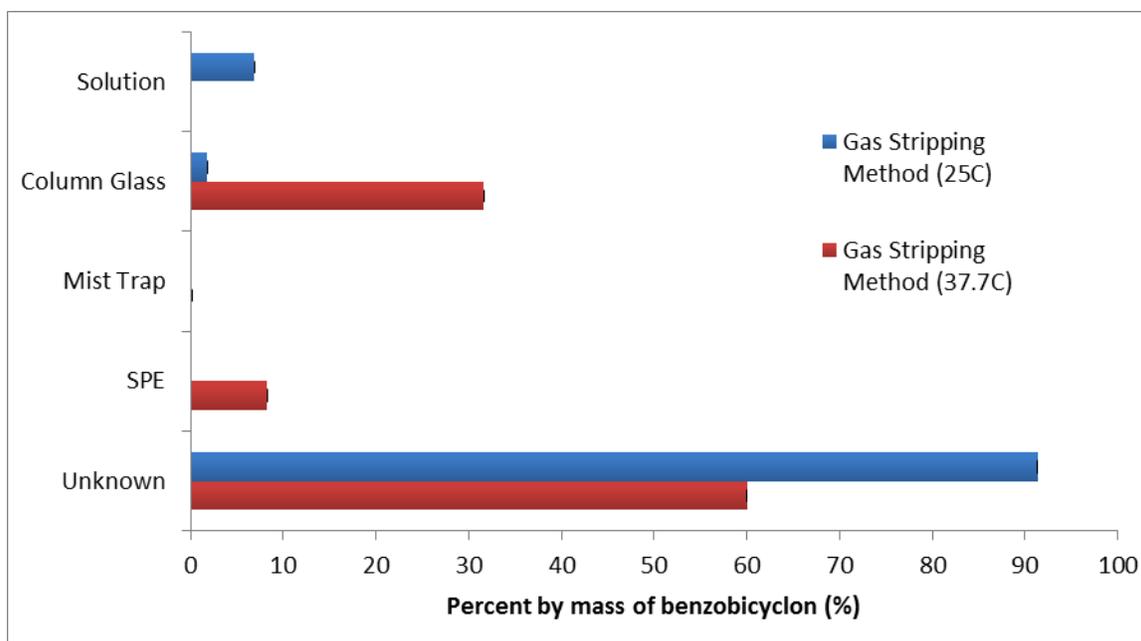
Figure 10: Mass distribution of Butte in the gas stripping columns.

under vacuum for 15 min, cartridges were rinsed with 1.5 mL of acetonitrile, which was collected and analyzed via HPLC-MS/MS.

After the aqueous Butte solution was drained from the apparatus the columns were rinsed with dichloromethane to remove any herbicide sorbed to the glass walls. The solvent was collected and reduced to near dryness via N_2 gas (30°C). Acetonitrile was used to reconstitute the samples; it was collected and analyzed via HPLC-MS/MS.

SPE cartridges were collected and volatilized Butte extracted using 1.5 mL of acetonitrile. These samples were analyzed via HPLC-MS/MS using an Agilent 1100 LC with PE Sciex API 2000 mass spectrometer (with electrospray ionization source, ESI). The analytical column was a Restek Allure C18 column (250 mm, 4.6 mm x 5 μm), and the mobile phases were 5 mM ammonium acetate acidified with 0.1% acetic acid (A) and acetonitrile (B); an isocratic method of 30% A and 70% B was used. The total run time was 16.5 min, with a 0.5-minute equilibration time. The injection volume was 25 μL and the flow rate was 0.6 mL/min. The method limit of detection was 4.4 ppb and the method limit of quantification was 10.6 ppb. The mass spectrometer was run in MRM mode with the Q1 mass for benzobicyclon set at 446.9 amu and the Q3 mass quantifying ion set at 256.9 amu.

Preliminary Results and Discussion

The gas stripping method was used to determine Butte's experimental Henry's Law constants at 25°C and 37°C. Figure 9 shows the decrease in aqueous Butte over time in both experiments. After 24 h, Butte was no longer detected in the 37°C aqueous samples.
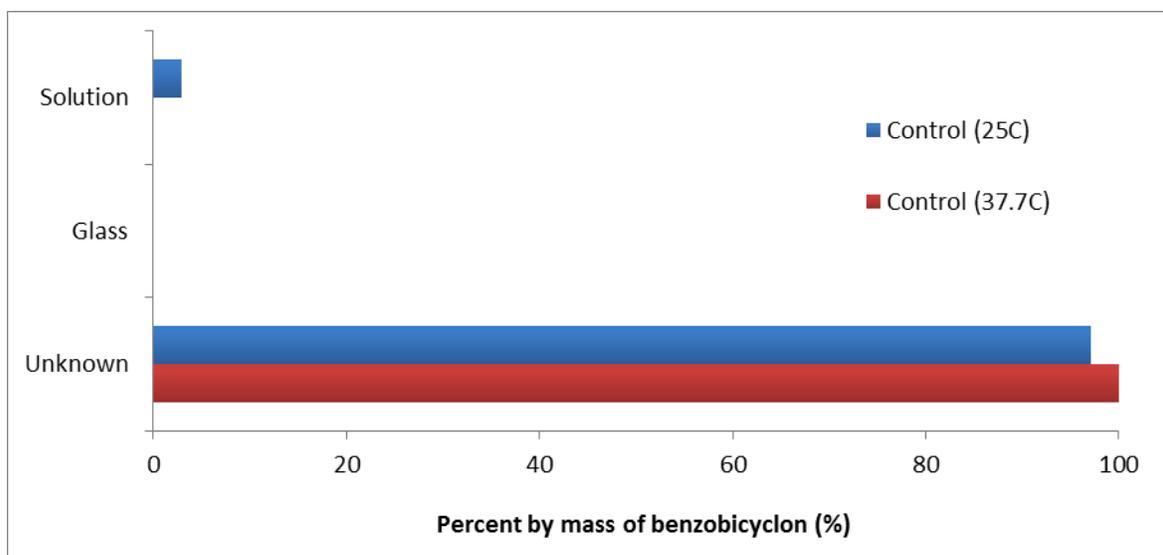
Figure 11: Mass distribution of Butte in the controls.

Mass distribution for each experiment was calculated by measuring the initial concentration of aqueous Butte (after equilibration) 3-fold, then averaging. This gave initial masses of 0.178 mg and 0.038 mg for the 25°C and 37°C experiments, respectively. The controls had a lower initial mass load compared to the columns (0.12 mg and 0.026 mg for the 25°C and 37°C experiments, respectively), as they held different volumes (1 L versus 1.5 L, respectively).

Column and mist trap rinses, and SPE cartridge extractions were also included as possible sinks for Butte during the experiments. The mass loads were normalized to the initial load in each column or control; results are in Figures 10 and 11. Approx. 60-100% (depending on the experiment) of the mass of Butte in the system disappeared by the end of each experiment. It is possible that Butte hydrolyzes rapidly in water to form BH, which may explain this. A hydrolysis study is underway to verify this.

The theoretical *H*s were calculated for both Butte and BH using the method described by Schwarzenbach *et al.* (2003). Aqueous solubility values for each were estimated at each temperature using AQUAFAC (Myrdal *et al.*, 1995).

Henry's Law constants have been estimated from the gas stripping method for Butte. Though the mass balance for each experiment was low, the control was able to account for the loss of Butte such that the constants could be calculated for the experiment. The experimental Henry's Law constants for BH will be determined next year, and fate work will then focus on BH – the active herbicide.

Table 1: Henry's Law constants for benzobicyclon and benzobicyclon hydrolysate.

Temperature	Gas Stripping Method		Theoretical Calculation[3]	
	25°C	37.7^{o}C	25°C	37.7^{o}C
Benzobicyclon	1.357 x 10^{-5} Pa m^3 mol^{-1}	2.811 x 10^{-3} Pa m^3 mol^{-1}	4.062 x 10^{-3} Pa m^3 mol^{-1}	2.028 x 10^{-2} Pa m^3 mol^{-1}
Benzobicyclon hydrolysate	TBD	TBD	7.447 x 10^{-10} Pa m^3 mol^{-1}	4.145 x 10^{-9} Pa m^3 mol^{-1}

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CONCISE GENERAL SUMMARY OF CURRENT YEAR'S RESULTS:

1. The overall goal of our ongoing research program is to characterize the dissipation of pesticides under California rice field conditions. There are generally four processes that can contribute to such dissipation that are investigated: volatilization to air, sorption (bonding) to soils, and degradation by either sunlight or soil microbes.

2. For the insecticide Belay, the capacity to sorb to rice field soils under standard conditions is low – due to its reasonably good water solubility – and is directly correlated to organic matter content.

3. For the herbicide League, preliminary data suggests that rapid degradation is observed in irradiated samples, and both light absorption and turbidity may influence photolysis rates. Hydrolysis was slower than photolysis, but not negligible.

4. For the herbicide Butte, complete results indicate that its ability to dissipate from rice field water via volatilization is minimal.