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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 natural factors governing the dissipation of pesticides in California rice fields. Emphasis for 2009 was to complete characterization of the microbial degradation of etofenprox under California rice field conditions.

Objective II. To investigate the natural factors governing the dissipation of pesticides in California rice fields. Emphasis for 2009 was to characterize the microbial degradation of Cerano (clomazone) under California rice field conditions, and to assess the role abiotic factors.

Objective III. To investigate the natural factors governing the dissipation of pesticides in California rice fields. Emphasis for 2009 was to complete characterization of the volatilization of clothianidin under California rice field conditions.

Objective IV. To investigate the natural factors governing the dissipation of pesticides in California rice fields. Emphasis for 2009 was to characterize the field dissipation of Cerano (clomazome) in a representative California rice field **(later add-on field study).**

SUMMARY OF 2009 RESEARCH (MAJOR ACCOMPLISHMENTS) BY OBJECTIVE:

OBJECTIVE I

Introduction

The partitioning of etofenprox between air, water and soil under representative California rice field conditions has been previously characterized. In summary, volatilization was shown to be an insignificant dissipation pathway as compared to soil sorption. Calculated Henry's Law Constant (*H*) values ranged from 5.6×10^{-3} Pa · m^3 /mol at 5°C to 2.9×10^{-1} Pa · m^3 /mol at 40°C

based on estimated solubility and vapor pressure values at various temperatures. In addition, etofenprox was found to very tightly sorb to two representative rice field soils (Princeton and Richvale), with log K_{oc} values of 6.0 and 6.4, respectively; sorption did not significantly differ between 25 and 35ºC (Vasquez *et al.*, 2009). Thus, although the insecticide will quickly dissipate from field water after application, it has the potential to remain in soils for an extended period.

Degradation via soil microbes represents one of the most important dissipation pathways for pesticides; it can greatly reduce an agent's persistence in a soil-water matrix, such as a rice field. Management practices in rice production create two soil types: anaerobic, when a field is flooded and aerobic when it is not. Thus, it is necessary to investigate soil communities for their activities under both conditions by measuring degradation rates and products. It was hypothesized that microbial communities endemic to California rice fields can efficiently degrade etofenprox. The temporal change in available oxygen has been shown to change the microbial community structure (Liesack, 2000). It was also hypothesized that the rate will be greater under aerobic versus anaerobic conditions. This study aimed to describe the microbial degradation of etofenprox under simulated rice field conditions by determining the dissipation rate constant (k) and half-life ($t_{1/2}$), and the degradation pathways under both anaerobic and aerobic conditions.

Materials and Methods

Chemicals, soil and irrigation water. Etofenprox, α-carbonyletofenprox (α-CO) and 4-hydroxyetofenprox (4'OH) were supplied *gratis* by Mitsui Chemical Co. (Japan). Sodium azide and 3-phenoxybenzoic acid (3-PBA) were purchased from Sigma Chemical Co. (St. Louis, MO). Stock solutions of etofenprox were prepared in methanol and used to prepare microcosm samples. Rice field soil was collected from the Rice Experiment Station (Biggs, CA) in May 2008; it was air-dried and ground to pass through a 2-mm sieve, then stored at 4°C until used. Water (pH 7.5) used for "flooding" the microcosms was collected from an irrigation outfall (Berryessa Irrigation District) located at UCD in July 2008.

Soil characterization. The soil is currently undergoing complete characterization by the Division of Agriculture and Natural Resources (ANR) Analytical Laboratory at UCD. Complete description of the methods is in Schmelzer *et al.*, 2005, or the ANR website (http://danranlab.ucdavis.edu).

Anaerobic microbial degradation of etofenprox. Microcosms were constructed with a 2.5 cm soil layer (50 g) and flooded to a depth yielding a 1.5 cm water layer (60 mL) in a 300 mL amber screw cap bottle. All microcosms were prepared in triplicate and incubated at room temperature in the dark. Control soils (triplicate) were autoclaved before flooding and application of etofenprox. Control "flood" water contained 200 mg/L sodium azide for microbial inhibition. The oxidation-reduction (redox) potential of the flooded soil (Figure 2) was monitored to determine when anaerobic conditions were achieved, which were generally within 14 days of flooding, thus this time interval was adequate for establishment of anaerobic conditions prior to introduction of etofenprox. Periodic soil sampling (t=0, 1, 3, 7, 14, 21, 28, 35, 42, 56 and/or 126 days) after etofenprox addition (150 ug) was used to determine degradation rates and metabolite formation. Microcosm samples were solvent extracted and then analyzed by LC/MS/MS.

Aerobic microbial degradation of etofenprox. Microcosms were constructed with a 2.5-cm soil layer (50 g) in 125-mL glass serum bottles. Bottle tops (uncapped) were covered with foil. Soils were maintained at approx. 40% moisture holding capacity using irrigation water over course of experiment. Irrigation water for controls included the biocide (sodium azide). All microcosms were prepared in triplicate and incubated at room temperature in the dark. Control soils (in triplicate) were autoclaved three times and etofenprox was applied to sterilized soils. Periodic sampling of the soil (t=0, 1, 3, 7, 14, 21, 28, 35, 42, and 56 days) after etofenprox addition (150 ug) was used to determine degradation rates and products over the course of the experiment.

Extraction and Analysis. To each microcosm, acetone (60 mL) was added and then placed on a platform shaker (135 rpm) for 24 h. The sample was then vacuum-filtered and the cake was washed with acetone. For anaerobic samples, the acetone was removed from the acetone-water extract by evaporation under N_2 gas. The remaining aqueous phase (after evaporation) was liquid/liquid extracted three times with hexane (20 mL each). The combined extracts were then dried with sodium sulfate and subsequently brought to dryness under N_2 gas. The residue was dissolved in 2 mL of 40:60 acetonitrile:water, filtered (0.45 um) and then analyzed by LC/MS/MS. For aerobic samples, water (50 mL) was added to the extract before evaporation under N_2 gas. The remaining aqueous phase was then extracted in the same manner as the anaerobic samples.

LC/MS/MS analysis of etofenprox and metabolites was performed using a HP 1100 HPLC (Palo Alto, CA) coupled to an Applied Biosystems Sciex 2000 triple quadrupole LC/MS/MS (South San Francisco, CA) using electrospray ionization (ESI) in positive mode. Chromatographic and MS/MS parameters are summarized in Table 1. Etofenprox, α-CO and 4'OH were quantified in multiple-reaction monitoring mode (MRM) against a second-order calibration curve generated in Analyst software version 1.2.4 using matrix matched standards. The instrument detection limit is 0.01 mg/L for etofenprox, 0.05mg/L for α-CO and 0.05mg/L for 4'OH and the extraction efficiency of the method (% recovery) is 82%±15%.

Redox potential. The redox potential (E_h) of the soil was monitored to ensure anaerobic conditions were achieved before etofenprox was applied and maintained over the course of the experiment. Separate microcosm samples were prepared at least in duplicate. Measurements were taken using a calibrated redox electrode (Thermo, Waltham, MA). Anaerobic conditions were achieved within 14 days of flooding according to the redox data (Figure 1) and thus etofenprox was added to the system after 14 days of flooding.

Data Analysis Aerobic and anaerobic degradation rates constants were calculated based on first-order kinetics where the rate constant (k) is calculated from the equation:

$$C_t = C_o e^{-kt} \qquad (1)$$

and C_o is the initial concentration (ug/g) of etofenprox, C_t is the concentration (ug/g) at time t (days) and k is the first-order degradation rate constant. The half life ($t_{1/2}$) is calculated according to the equation:

$$t_{1/2} = (\ln ½)/k \qquad (2)$$

Table 1. (A) Selected-ion transitions for etofenprox and metabolites using positive mode ESI LC/MS/MS; sum of ions used for analyte quantification. (B) Chromatographic conditions using Titan C18 column (5 um, 2.1 mm ID x 100 mm), 0.25 mL/min flow rate, and 40-uL injection.

Table A:

Transition Monitored (mass/charge)	Analyte, MW	Q1 and Q2 parameters DP/FP/EP/CE/CXP
394.4 to 359	Etofenprox 376	40/400/10/20/3
394.4 to 177.1	Etofenprox 376	40/400/10/20/3
359 to 183	Etofenprox 376	40/400/10/20/3
407.8 to 177.4	α-CO 390	30/400/10/19/3
407.8 to 134.9	α –CO 390	30/400/10/19/3
407.8 to 149.3	α –CO 390	30/400/10/19/3
409.8 to 177.1	4'-OH 392	40/400/10/55/3
409.8 to 134.9	4'-OH 392	40/400/10/55/3
375.1 to 177.1	4'-OH 392	40/400/10/55/3
203.3 to 93.2	3-PBA 214	40/400/10/55/3
237.2 to 81.0	3-PBA 214	40/400/10/55/3
149.2 to 81.0	3-PBA 214	40/400/10/55/3
Same as 4'OH	6'-OH 392	40/400/10/55/3
Declustering Potential (DP), focusing potential (FP), entrance potentail (EP), collision energy (CE), collision exit potential (CXP), multiple reaction monitoring (MRM)		

Table B:

Time (min)	Water (%) +0.1% ammonium acetate	ACN (%) +0.1% ammonium acetate
0	60	40
4.5	5	95
10	5	95
11	60	40
20	60	40

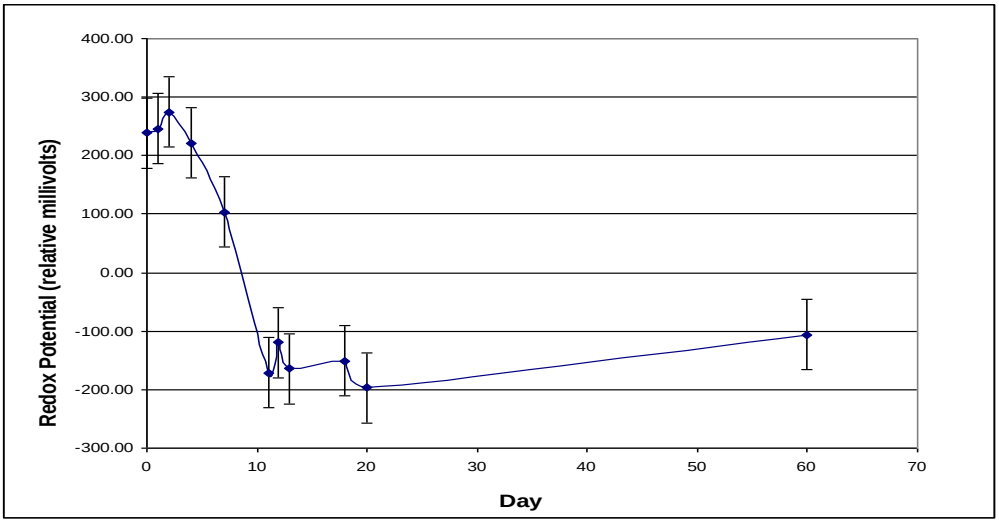


Figure 1. Redox profile of flooded soil at various times. Anaerobic conditions (-100 to -200 rmV) achieved within 14 days of soil flooding.

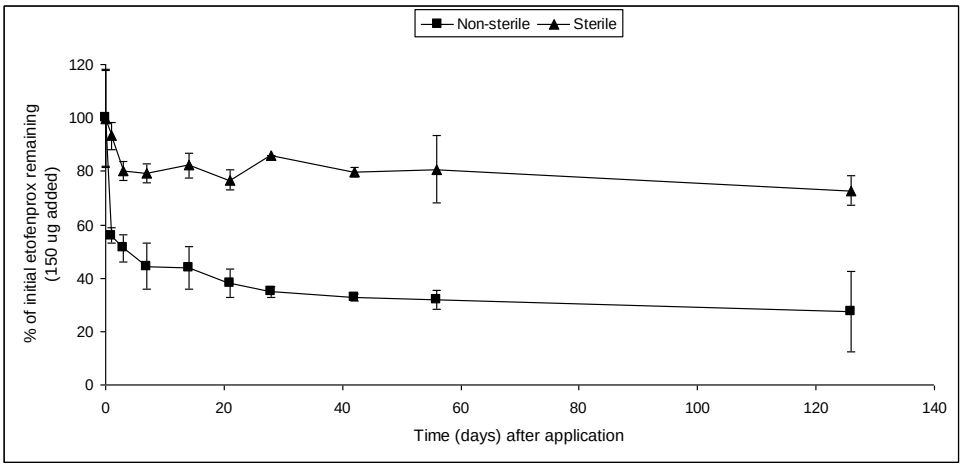


Figure 2. Dissipation of etofenprox in a flooded (anaerobic) soil under both non-sterile (■) and sterile (▲) conditions; points represent average (n=3) ± SD.

Results/Discussion

Etofenprox dissipation under both anaerobic and aerobic soil conditions showed a fast initial decrease (0 to 3 days) in the percent-applied mass (Figures 2 and 3), followed by a slower decrease as the time course progressed; controls showed little degradation over the course of the experiment. The pseudo-first order dissipation kinetics of etofenprox in the anaerobic and aerobic California rice soils are summarized in Table 2. Etofenprox was more persistent in the anaerobic (flooded) soil (overall $t_{1/2}$ = 100 days, k = -0.0069 day^{-1}) compared to the non-flooded, aerobic soil (overall $t_{1/2}$ = 27 days, k = -0.0252 day^{-1}) at 22°C±2°C. The overall pseudo first-order dissipation rate was calculated using all the time points whereas the fast and slow rates were calculated using the 0 to 3 day points and the day 3 and onwards points, respectively (Table 2).

Similar $t_{1/2}$ values (fast dissipation kinetics) were found for both anaerobic and aerobic soils (3.5 days vs. 3.8 days, respectively), whereas the slow kinetic region of the dissipation curves results in very different $t_{1/2}$ values for the anaerobic and aerobic soils (154 days vs. 27 days, respectively). Due to the persistence shown for etofenprox under flooded conditions at 22°C ± 2°C, we are currently conducting the same experiments at 40°C to more closely mimic the extreme summer temperatures of the Sacramento Valley. It is hypothesized that degradation

Table 2. Summary of pseudo first-order dissipation kinetics of etofenprox under anaerobic (flooded) and aerobic (non-flooded) conditions.

	Aerobic at 22°C±2°C		Anaerobic at 22°C±2°C	
		r^2		r^2
Overall Rate Constant (k) (1/days)	-0.0252	0.7978	-0.0069	0.5081
Overall Half life (days)	**27.5**		**100**	
Slow Kinetics Rate constant	-0.0182	0.8801	-0.0045	0.7629
Slow kinetics half life (days)	38.1		154	
Fast Kinetics rate constant	-0.1839	0.9768	-0.1967	0.6873
Fast kinetics half life (days)	3.8		3.5	

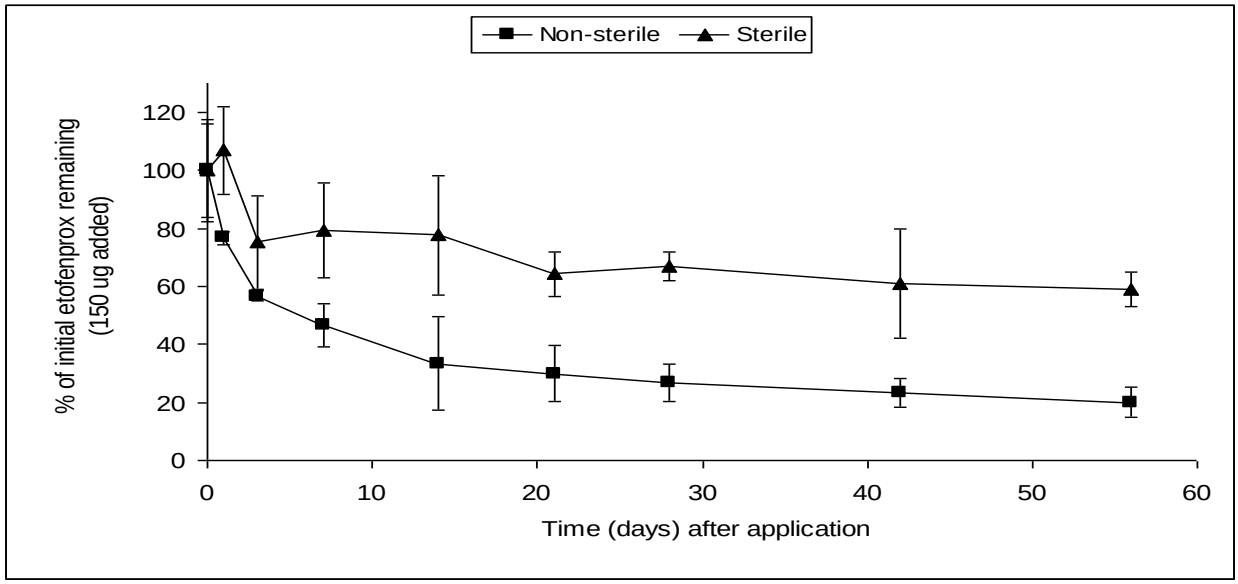


Figure 3. The dissipation of etofenprox in a non-flooded (aerobic) soil over time under both non-sterile (■) and sterile (▲) conditions. Points represent mean ± SD (n=3).

and dissipation will be faster at a higher temperatures – thus persistence will be decreased.

Two metabolites were identified under anaerobic conditions (Figure 4), 4'-OH (maximum yield 1.34 ± 0.65% of total etofenprox applied) and α-CO (max. yield 0.93 ± 0.38%). The same two main metabolites, 4'-OH (max. yield 0.26 ± 0.05%) and α-CO (max. yield 0.51 ± 0.09%), were detected under aerobic conditions but in lower amounts relative to anaerobic samples (Figure 5). An unidentified aerobic metabolite was detected with the same ion transitions as 4'OH but with a shorter retention time; it was not present in anaerobic samples. The hydrolysis product of α-CO, 3-phenoxybenzoic acid (3-PBA), was not detected in either anaerobic or aerobic samples.

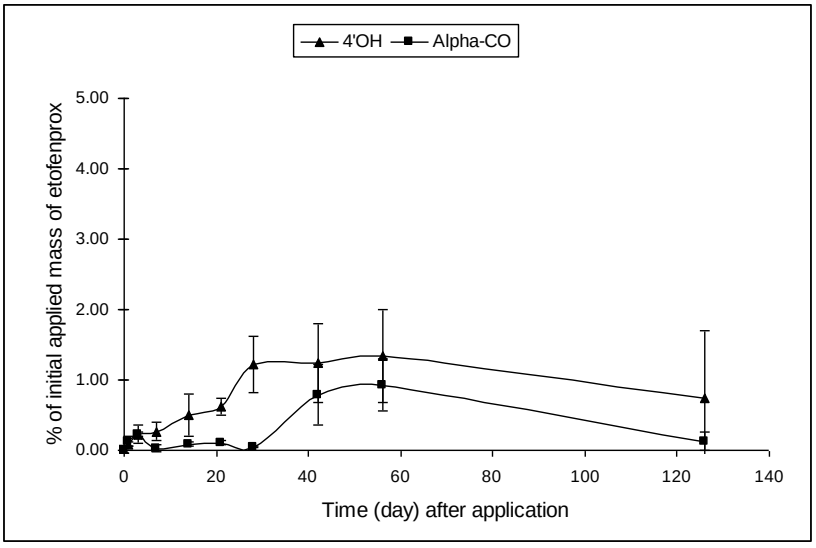


Figure 4. Major etofenprox metabolite formation under anaerobic (flooded) soil conditions: 4'OH (▲) and α-CO (■). Points represent mean ± SD (n=3).

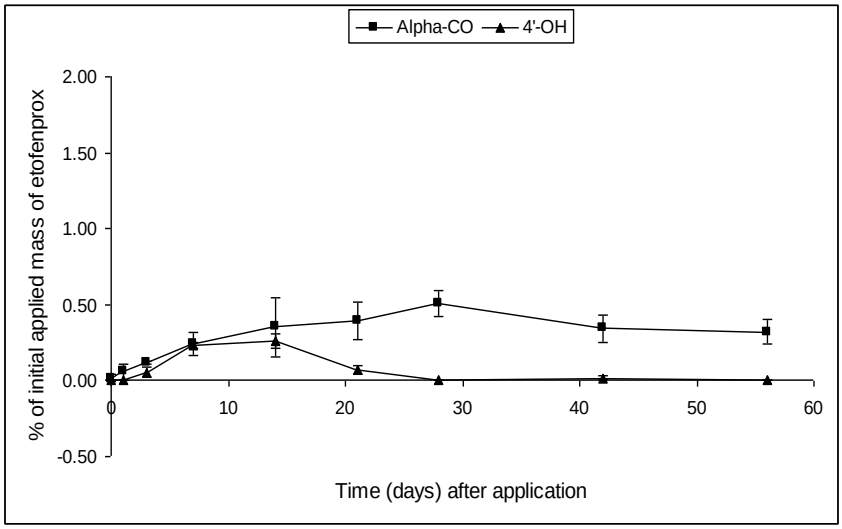


Figure 5. Major etofenprox metabolites formation under aerobic (non-flooded) soil conditions: 4'OH (▲) and α-CO (■). Points represent mean ± SD (n=3).

This is possibly due to lack of acidification before hexane extraction (and thus 3-PBA did not partition into the organic layer) as to not hydrolyze the α-CO already in the sample extract.

The major metabolite under anaerobic conditions was 4'OH, whereas that under aerobic conditions was α-CO, suggesting different pathways of degradation under different oxic conditions. A suggested pathway for etofenprox degradation under both conditions is presented in Figure 6. In a reductive environment, such as a flooded rice field, O_2 is not available for use as an electron acceptor for microbial energy production, nor is it available as an oxygen donor for microbial cytochrome P450-mediated reactions. Oxidation to an ester of an ether linkage is a common P450-mediated reaction in the oxidative environment (Crosby, 1998), but less common

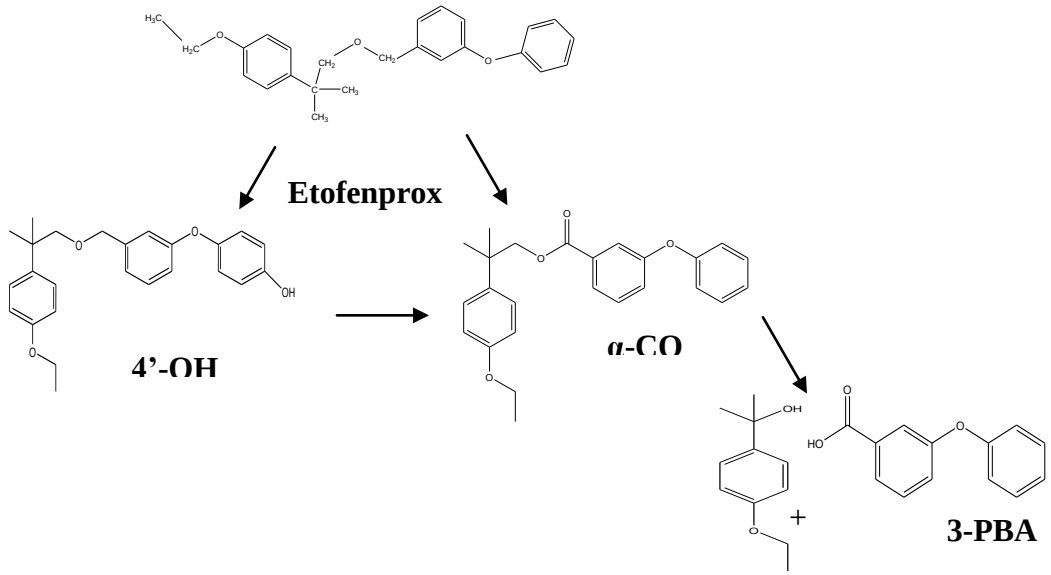


Figure 6. Proposed degradation pathway for etofenprox based on known metabolite formation and structures.

in an anoxic environment lacking O_2 as substrate. Hydroxylation under anaerobic conditions may result from water as the oxygen donor in an enzyme-catalyzed reaction mediated by microbes. Its occurrence would support the production of greater quantities of 4'-OH (relative to the amount of α-CO) under anaerobic versus aerobic conditions.

Microbial populations in both flooded and non-flooded California rice soils were able to effectively transform etofenprox, thus contributing to its dissipation. Due to the persistence of etofenprox under anaerobic conditions ($t_{1/2}$ = 100 days), we are currently conducting similar degradation experiments at 40°C.

Objective II.

Cerano (clomazone; 2-[2-chlorobenzyl]-4,4-dimethyl-1,2-oxazolidin-3-one) is an herbicide used on California rice fields to control watergrass, barnyard grass and sprangletop, and is used elsewhere in the world for many other crops, notably soybeans, cotton and tobacco (CDPR, 2003). Cerano was registered for use in California in 2002 and, as of 2007, has increased to 80,000 lbs active ingredient (a.i.) applied to 160,000 acres (CDPR, 2007), making it one of the most popular rice herbicides in California. In plants, its mode of action is thought to occur via cytochrome P450 activation followed by carotenoid synthesis inhibition (TenBrook *et al.,* 2006; Mueller *et al.*, 2000; Ferhatoglu *et al.*, 2006). Cerano is strongly water soluble, weakly sorptive, minimally volatile and resistant to hydrolysis under a range of pHs (Senseman, 2007). These physical-chemical characteristics indicate Cerano is likely to persist primarily in the water column, where microbial degradation may contribute to its environmental fate.

Microbes oxidize organic compounds in the presence of terminal electron acceptors, and reduce them in a sequential series dependent upon redox potential, E_h, as alternative electron acceptors are utilized (Sposito, 1989). Flooded rice fields are a mosaic of anaerobic soil with aerobic micro sites, and under these conditions Cerano's degradation rate and metabolite identities may differ. Though knowledge of specific colonies involved in degradative processes would serve helpful, knowing soil degradation kinetics is paramount to determining how long the fields should be held prior to draining.

The objectives of this study were to: 1) determine the kinetics of Cerano microbial degradation, and 2) determine the main metabolites formed under aerobic and anaerobic conditions. Experiments were conducted via sacrificial time-series microcosms at 30°C, typical of summertime highs in the Sacramento Valley. Microcosms consisting of autoclaved water and soil sampled from the Biggs Rice Research Station were extracted over 83 days; controls were autoclaved. At defined time intervals samples were sequentially extracted with water and acetonitrile to mimic water-dissolved and soil-bound fractions, respectively, and analysis was via LC/MS/MS.

Under anaerobic conditions Cerano degraded rapidly, with a half-life of 7.9 days. Ring-open Cerano (RO) was the only metabolite observed in significant amounts (>1% Cerano application). RO reached 67.4% abundance at 38 days. Under aerobic conditions Cerano degraded more slowly, with a half-life of 47.3 days (Figure 1); no aerobic metabolites were observed in significant amounts. Trace levels of 5-OH and ArOH were observed in anaerobic samples, and 5-

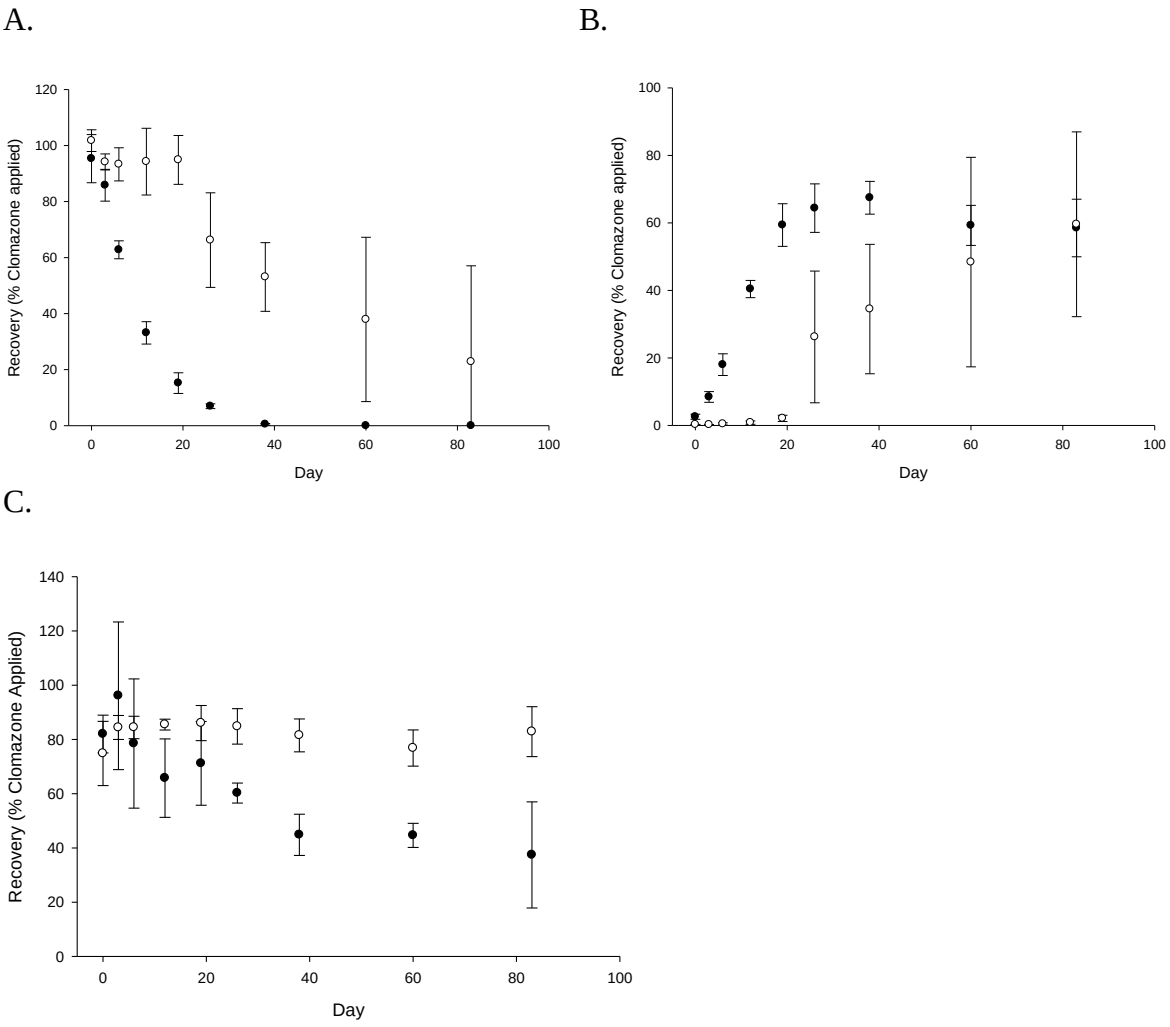


Figure 1. Percent recovery of Cerano and RO (expressed as percent of Cerano-applied) for treated soil (solid points) and autoclaved controls (open points) under anaerobic conditions (A), showing anaerobic ring-open formation (B), whose recovery is expressed relative to Cerano, and under aerobic conditions (C). Data points are means ± 1 SD (controls, n =3; treatments, n = 5).

OH, ArOH, and 4'5-diOH found in aerobic samples. All products of hydroxylation were observed below quantification limits, constituting <1% of application amount. No other metabolites were found in either the water or acetonitrile fractions at concentrations greater than 0.02 µg/mL.

From these results, we believe anaerobic degradation in rice fields is likely to significantly contribute to Cerano's dissipation. The herbicide degraded rapidly to RO under anaerobic conditions but only slowly aerobically, mostly forming soil-sorbed residues. In rice fields, as soil redox potential decreases, Cerano degradation and RO formation rates are expected to increase. These transformations are microbially mediated, as confirmed by comparison with autoclaved controls. This investigation provides farmers and regulators alike with information necessary to make informed decisions on Cerano management.

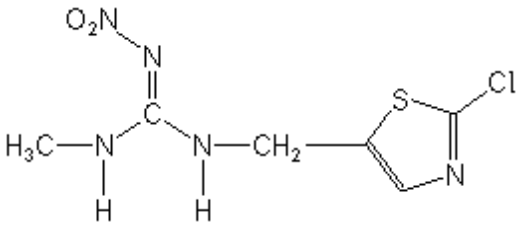


Figure 1. Structure of clothianidin.

Objective III

Clothianidin, (E)-1-(2-chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine (Figure 1) is a novel neonicotinoid insecticide that exhibits a good systemic action and high insecticidal activity against various sucking insect pests (Umene *et al.*, 2006). It has been registered for foliar-spray and seed-treatment applications for food crops in various countries throughout Europe and Asia (Franklin *et al.*, 2004), and is of a current interest for rice culture in California due to its effectiveness against the water weevil. Due to its efficient mode of action (involving cholinergic properties of the central nervous system), neonicotinoids show no cross-resistance to conventional insecticide classes and thus have begun replacing established classes such as the pyrethroids, chlorinated hydrocarbons, organophosphates and carbamates (Jeschke *et al*., 2008).

Two chemodynamic mechanisms are of primary importance when considering the abiotic dissipation of an insecticide from rice fields: 1) air-water partitioning, and 2) soil-water partitioning. The first describes the potential volatility of the agent, or more specifically its Henry's law constant (*H*), since air-water partitioning is dependent on both water solubility and apor pressure, as compared by the constant (Schwarzenbach *et al.*, 2003). This past year we investigated the potential volatility of clothainidin. Henry's law constants were measured via standardized methods and California rice field conditions (representative temperatures, i.e. 20 and 40°C), varied water retention periods (i.e. 24 and 48 h), and the dynamic equilibrium approach of Schwarzenbach *et al.* (2003). Air-water partitioning can contribute significantly to field dissipation, and is influenced by managed water retention time (Mabury *et al.*, 1996).

Materials and Methods

Estimation of Henry's law constant by group contribution method. When an agent's physical-chemical properties are not available at the temperature of interest, they can be estimated by using the chemical structure. Two properties, vapor pressure and aqueous solubility, were estimated in order to calculate the Henry's law constant (*H*) for clothianidin. For vapor pressure, the boiling point of clothianidin was first determined by the group contribution method described by Stein and Brown (1979), which was subsequently used to estimate the vapor pressures at temperatures ranging from 5-40 °C using the Kistiakowsky-Fishtine equation (Schwartzenbach *et al*., 2003). The aqueous solubility of clothianidin at the various temperatures was estimated using the AQUAFAC group contribution method described by Myrdal *et al*. (1995). The Henry's law constant of clothianidin was calculated by dividing vapor pressure by aqueous solubility at a given temperature.

Table 1. Solvent gradient program for LC/MS analysis of clothianidin aqueous samples.

Time (min)	Water (%)	Methanol (%)
0.00	95	5
20.00	5	95
20.01	95	5
25.00	95	5

Table 2. Detailed mass spectrometer parameters for LC/MS/MS analysis.

Parameters	Clothianidin m/z 250.3 → 169.3
Gas	Nitrogen
Curtain Gas (L/min)	20
Collision Gas (L/min)	4
Ionspray Voltage (V)	5500
Temperature (°C)	375
Ion Source Gas 1---Nebulizer (L/min)	30
Ion Source Gas 2---Turbo Gas (L/min)	0
Declustering Potential (V)	26
Focusing Potential (V)	360.0
Entrance Potential (V)	11.0
Collision Energy (V)	19.0
Collision Cell Exit Potential (V)	6.0

Experimental determination of Henry's law constant. This was performed by the gas-stripping apparatus method (Mackay *et al*., 1979). Custom-made gas stripping columns (1 m x 51 mm id Pyrex cylinders) containing approx. 1.5 L of a 0.5 mg L^{-1} clothianidin solution in 0.01 M $CaCl_2$ were run in duplicates. The columns were wrapped in aluminum foil to prevent photodegradation of the analyte. Measurements were taken at two different temperatures, 20 and 40°C, and the column temperature was maintained by a recirculating water bath. A stream of N_2 gas (filtered through a hydrocarbon trap and saturated with water) at a flow rate of 1000 mL/min was introduced to the column through a tube insert located at the bottom of the cylinder. Aqueous samples (10 mL) were collected at various time intervals over the course of the experiment (48 h) to measure the disappearance of the agent from the solution. Collected samples were filtered using a 3-mL syringe with nylon syringe filter (13 mm, 0.2 µm), then stored in sealed amber glass HPLC vials at 4°C for later analysis.

LC/MSMS Analysis. Aqueous samples were analyzed via an HP Series 1100 HPLC (Hewlett Packard, Inc.) coupled with API 2000 LC/MS/MS System (Applied Biosystems, Inc.). A Titan C_{18} analytical column (5 um particle size, 2.1 mm id x 100 mm; Peeke Scientific) was used at ambient temperature, via solvent gradient, at a flow rate of 0.25 mL/min (Table 1). Injection volume was 20 µL, using an HP Series 1100 Autosampler, and the column eluent was directed to the MS for a run of 6 to 20 min. Positive-ion electrospray ionization was used with optimized MS parameters (Table 2), and the ion transition (m/z 250.3→169.3) of clothianidin was acquired

Table 3. Estimated physical and chemical properties of clothianidin at various temperatures.

Physical Chemical Property	Estimated Value	Manufacturer Reported Value
Boiling Point	351.52 °C	N/A
Vapor Pressure (Pa)		
5 °C	2.41×10^{-11}	
15 °C	6.96×10^{-11}	
20 °C	1.15×10^{-10}	3.8×10^{-11} [a]
25 °C	1.87×10^{-10}	1.3×10^{-10} [b]
35 °C	4.71×10^{-10}	
40 °C	7.30×10^{-10}	
Aqueous Solubility (g/L)		
5 °C	0.36	
15 °C	0.45	
20 °C	0.51	0.327 [a]
25 °C	0.57	
35 °C	0.72	
40 °C	0.80	
Henry's law constant (Pa-m^3/mol)		
5 °C	7.19×10^{-12}	
15 °C	1.60×10^{-11}	
20 °C	2.32×10^{-11}	2.9×10^{-11} [b]
25 °C	3.30×10^{-11}	
35 °C	6.41×10^{-11}	
40 °C	8.72×10^{-11}	

[a] reported by Sumitomo Chemical
[b] reported by Bayer

by multiple reaction monitoring mode. An 8-point calibration curve with linear regression was generated by potting the instrumental response of clothianidin for m/z 250.3→169.3 transition calculated with Analyst Software Version 1.4.2.

Results and Discussion

Group contribution method. The Henry's law constant (*H*) calculated via group contribution method was larger than the value reported by the manufacturer: 5.65×10^{-8} versus 2.9×10^{-11} Pa-m^3/mol at 20 °C (Table 3). The difference between the *H* values could have been due to the fact that the *H* value reported by the manufacturer was calculated using experimentally-determined vapor pressure and aqueous solubility while the physical chemical properties used in the group contribution method was determined solely by calculation. Despite the difference in the two values, both indicate that volatilization will not be the major dissipation pathway. In addition, the constant remained low over the range of 5-40 °C as shown on Table 3. Thus, volatilization of clothianidin is negligible for the range of temperatures typically found in California rice fields.

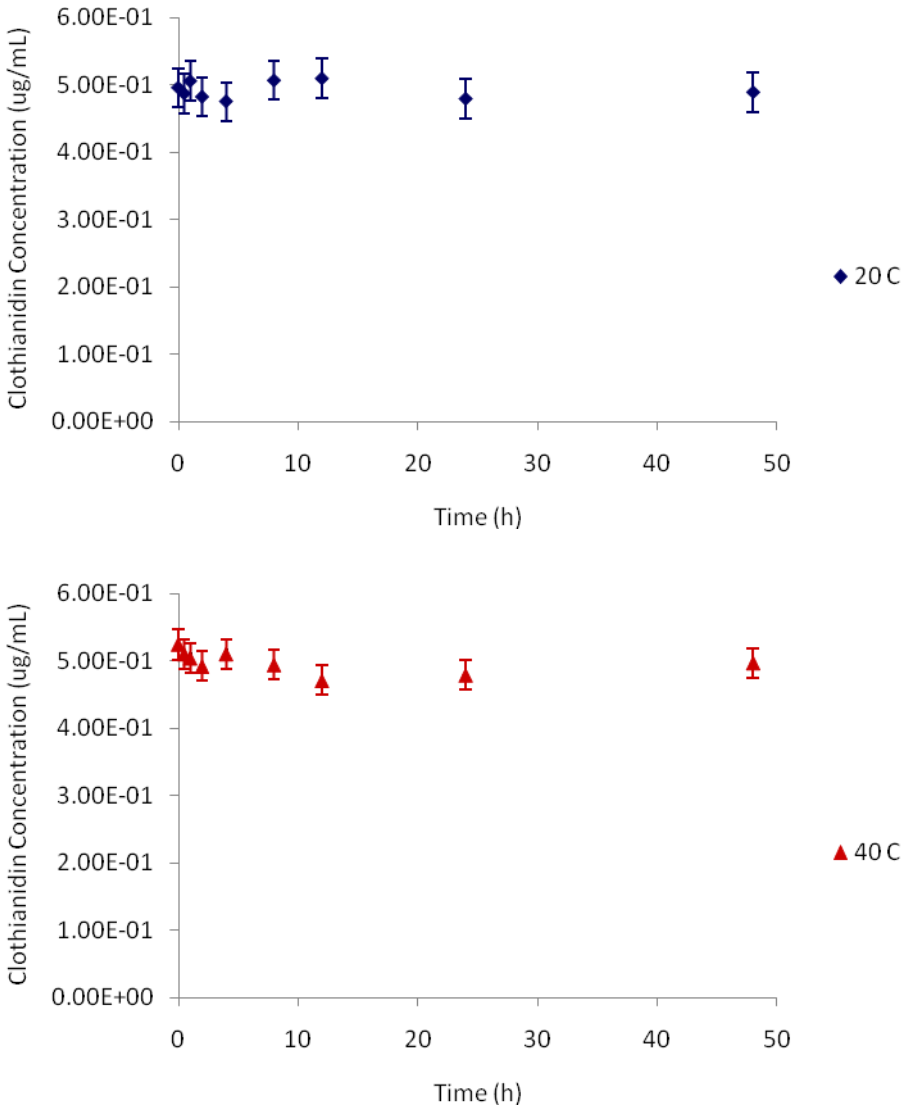


Figure 2. No distinct loss of aqueous clothianidin was observed in the gas-purge apparatus at 20 °C (top graph) and 40 °C (bottom graph). These results indicate the non-volatile nature of the insecticide. No detectable amount of clothianidin was found in the air phase.

LC-MS Analysis. MS parameters under optimum conditions for the analysis of clothianidin (Table 2) were determined by injecting a clothianidin standard (10 ppm) into the MS; the retention time was at 13.28 min. The transition at m/z 250.3→169.3, occurring due to cleavage of the nitro- and the chlorine-moieties from the parent compound under the optimized collision conditions was the most abundant transition for clothianidin and thus used to quantify the agent. The calibration curve correlation coefficient was 0.9987, and the limits of detection and quantitation for the instrument were 0.0103 ppm and 0.0329 ppm, respectively.

Gas-purge method. No decrease in aqueous clothianidin was observed in the gas-stripping apparatus at either 20 or 40°C (Figure 2). Mass distribution within the apparatus was investigated and no analyte was present in the air phase. As described by Mackay *et al*. (1979), when the aqueous phase is well mixed, the rate of mass transfer of analyte in aqueous phase into air phase

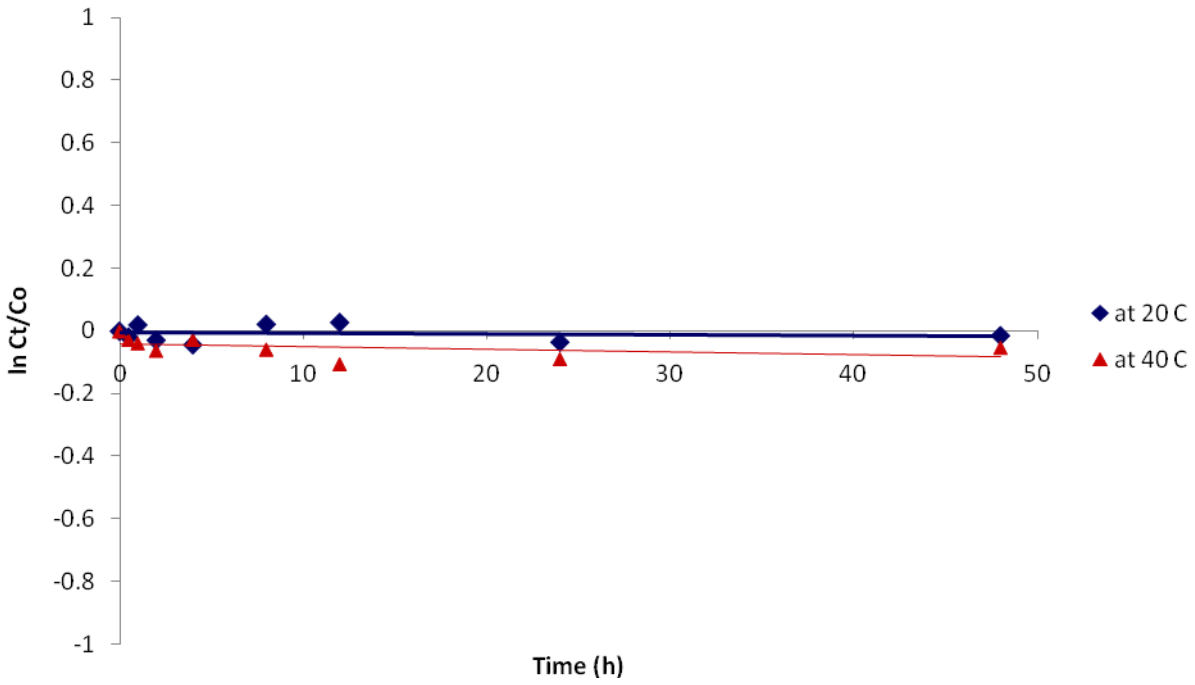


Figure 3. Ln Ct/C_0 vs. time was plotted in order to determine Henry's law constant of clothianidin. Experimental conditions were: T=293.15 K and 313.15 K, V= 0.0015 m^3, and G= 0.06m^3/h. The values of slope, -(HG/VRT), yielded from the plots at 20 °C and 40 °C, were -0.0002 and -0.0008, respectively.

follows the first-order decline in aqueous analyte concentration, shown as the equation below:

$$-V\, dC/dt = HGC/RT$$

where V is the volume of aqueous solution in m^3, C is the analyte concentration, t is the time in hours, H is the Henry's law constant in Pa-m^3/mol, G is the gas flow rate in m^3/h, R is the universal gas constant and T is the temperature of the system in Kelvin. Integrating this equation from initial condition when t=0 and $C=C_0$ gives:

$$\ln (C_t/C_0) = - (HG/VRT)\, t$$

The natural log of the aqueous clothianidin concentration over time was plotted (Figure 3). The resulting graph yielded slopes -0.0002 and -0.0008 for 20 and 40°C, respectively. A plot of log concentration against time yields a linear relationship with a slope of –(HG/VRT). However, because the aqueous concentration of clothianidin did not decline, no measurable volatilization occurred. Thus, Henry's constant values could not be measured due to the extremely low vapor pressure of the insecticide. Therefore, volatilization represents a negligible dissipation pathway for clothianidin in flooded ricefields.

Objective IV (add-on field study)

The Physical-chemical properties of Cerano can predict its fate once applied to flooded rice fields; they are summarized in Table 1 below.

Table 1: Physical and chemical properties of clomazome.

Properties	**Values**
Physical State	Crystalline Solid
Density	1.16 g/ml at 22^{o}C
Solubility (water)	1100 mg/L
Vapor Pressure	1.44 x 10^{-4} mm Hg at 25^{o}C
Octanol/water partition coefficient	352 at 23^{o}C
pH	8.6 at 25^{o}C
Storage stability	Stable one year at ambient temp

For instance, Cerano is highly soluble in water at 1100 mg/L, has a low *K*oc (organic carbon partition coefficient) of 150 mL/g, and a *K*d (soil sorption constant) ranging from 0.47 to 5.30 ml/g.[5] Prior Research has shown that pesticides may contaminate groundwater if they possess a water solubility greater than 30 mg/L, *K*oc below 300-500 mL/g, *Kd* less than 5 mL/g, and a soil half-life longer than 3 weeks.[5] Therefore, Cerano's properties suggest that it may be persistent in water, with a potential to contaminate groundwater. Cerano also has a moderate volatility, with a vapor pressure of 1.44 x 10^{-4} mm Hg at 25^{o}C.[4] Considering its Henry's law constant of 4.14 x 10^{-8} atm-m^{3}/mol, Cerano is expected to remain in the water column as opposed to volatilizing[4].

However, in the terrestrial environment Cerano may volatilize from soil. Vapor-phase transport and microbial degradation appear to be the major dissipation routes. Cerano is stable to hydrolysis in acidic, neutral, and alkaline solutions and does not photodegrade in either water or on soil. In soil, Cerano is metabolized under aerobic conditions with half-lives ranging from 28-173 days, depending on soil type; CO_2 is the major product. Under anaerobic conditions, Cerano readily degrades ($t_{1/2}$: 13 days) to (*N*-[(2-chlorophenol)methyl]-3-hydroxy-2,2-dimethyl propanamide), which persists under anaerobic conditions[4]. Volatility tends to increase with increasing temperatures, thus Cerano may volatilize from flooded rice fields and affect non-target crops. For this reason, it is important to study and understand the dissipation and degradation of Cerano from flooded rice fields[4].

To minimize environmental impacts, Cerano-treated water should be contained on the rice field for a minimum of 14 days following application.[1] This allotted time implies that Cerano should be virtually dissipated from the rice field within 14 days. Dissipation during this period is also important in regards to drinking water quality. EEC Directive 80/778 establishes the maximum permissible concentration of Cerano as 0.1 ug/L (ppb). And since the detection of most organic compounds in surface water is 1-3 ppb, the enforcement of the drinking water standard requires analytical methods with high sensitivity, selectivity, accuracy, and precision.[7] Thus for this study, a multistep process involving field sampling, sample preparation using SPE and analysis

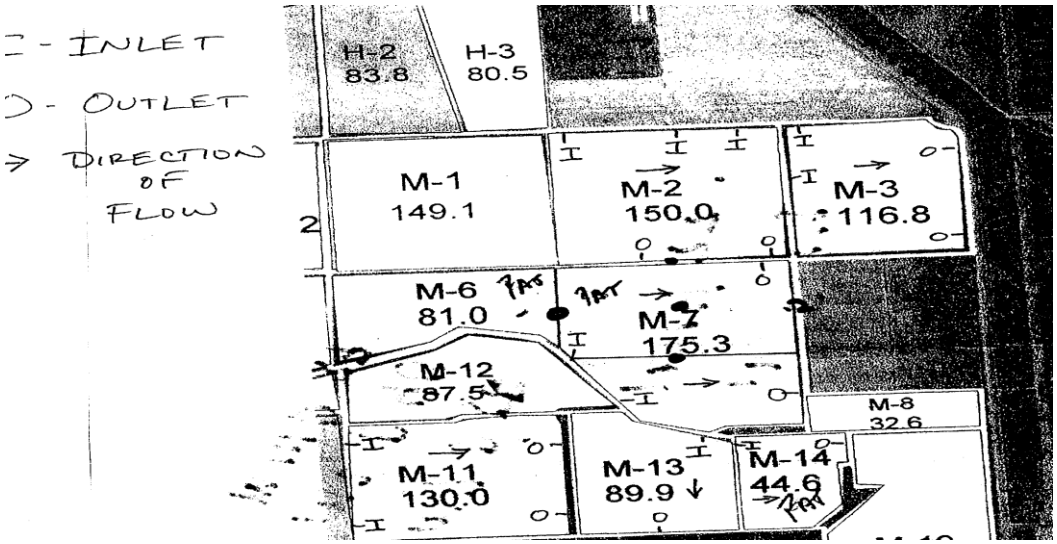


Figure 1. Location of each field studied (M2, M7 and M14) along with the acreage and direction of water flow.

of Cerano using HPLC-DAD was implemented to monitor the dissipation characteristics from a California rice field.

Materials and Methods

Commercial rice field. Samples were collected from an 1100 acre farm, managed by M. Daddow, containing 10 separate fields (Davis, California). This site had fairly high salinity (500 ppm TDS, pH > 8). For the purpose of this study, 3 replicate fields were examined; M2, M7 and M14. Application of Cerano began in early May for M7, mid-May for M2 and late May for M14 (Figure 1), and sampling was as follows: days 1, 2, 3, 4, 5, 7, 10, 12, 14, 18, 20, 24, 29, 32, 34, 37, 40, 45, 50, 55, 65, 75, 80, 85 and 90.

After the fields were flooded, the irrigation system was held static for fourteen days, and then water from the irrigation canal was allowed to flow into the flooded fields through the inlet. Water depth was held within a range of 2 to 6" (with an approx. average of 4"), depending upon the location within the field (Figure 1). Cerano 5 MEG® (containing 5% active Cerano) was applied at the minimum of 8 lb/acre in order to minimize rice crop damage that may result with higher concentrations mixed with high salinity and pH (as previously noted). The herbicide was applied aerially across each field and thus a theoretical initial concentration was calculated at 441 ppb (using average water depth) assuming that it was evenly distributed. A theoretical maximum initial concentration range was also calculated using water depths of 4, 5, and 6", corresponding to concentrations of 441, 353, and 294 ppb, respectively.

Chemicals and reagents. Cerano was obtained from FMC Corporation (Philadelphia, PA). Methanol and acetone were obtained from Fisher Scientific (Missouri, USA) and Sigma Aldrich (New Jersey, USA), respectively. Phosphoric Acid (85% assay) was obtained from Merck, Germany. Milli-Q water was purified using a Barnstead Ultrapure water system (Iowa, USA). The extraction cartridges were Bakerbond™ SPE Octadecyl (C-18) Disposable Extraction Columns (Kentucky, USA).

Instrumentation. Analysis was via an Agilent 1200 Series HPLC with autosampler and quaternary pump coupled with an HP Series 1100 diode-array detector (220 nm). A stock standard solution of 1010 ppm was prepared in mobile phase (MeOH: H_20) and used to prepare calibration standard concentrations. An isocratic mobile phase was implemented, consisting of a 65:35 mixture of methanol and water, adjusted to a pH of 4 using concentrated phosphoric acid. It was prepared from separately-measured volumes of methanol and water. Mobile phase pH was measured with an Accumet® portable AP61 pH meter (Fisher Scientific, USA). The flow rate was set to 1.0 mL/min and was held static during a seven minute run time. Analyte (60 µL) was injected into the HPLC and retained on an Agilent Eclipse XDB-C18 column (4.6 mm x 150 mm with 5-um diameter packing). Mobile phase was allowed to pass through at 1 mL/min to condition the column for 1 h before performing analysis of the samples. Limit of detection was 0.022 mg/L and the % RSD for seven replicate injections of a 1.01-ppm calibration standard solution was 0.00096%.

Procedure. Water samples were taken using 1-L amber bottles cleaned with detergent and 10% HCl and methanol. Each bottle was conditioned before collection by rinsing with at least 100 mL of field water three times (discarded after each rinse). Each bottle was then filled completely to minimize headspace, capped, and stored in an insulated ice chest until transported to the laboratory. Approximately 30 mL of each sample was transferred from the amber bottle into a vial for storage at -20° C. Soil samples were collected from the same sites using an auger. The top 8 cm of soil were transferred to polyethylene bags and stored in an insulated ice chest, transferred to the laboratory, and placed in a freezer for storage at -20° C for about 3 mo prior to analysis. Both water and soil samples were taken from three locations on M2 and M14; inlet, middle, and outlet. Four samples were taken from field M7; inlet and outlet for N and S, while three samples were taken from M2 and M14 (Figure 1).

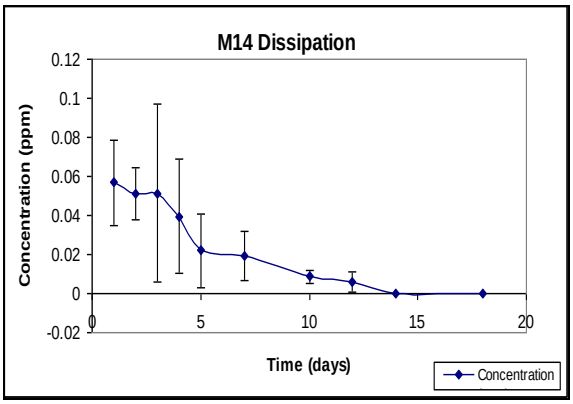
Water samples were filtered through a 0.22 um filter unit to remove particulate matter. The C-18 SPE cartridge was conditioned (at 3 mL/min flow rate) with 3 mL methanol, 3 mL Milli-Q water, and 3 mL Milli-Q water adjusted to pH 3 with phosphoric acid. A 30-mL aliquot of water sample was then applied to the cartridge, and immediately after the cartridge was washed with 3 mL of Milli-Q water to remove water-soluble impurities. It was then dried under vacuum for 2 min and eluted with 1 mL of methanol. The solvent was evaporated to dryness under N_2 gas, re-dissolved in 0.5 mL of mobile phase (this amounted to a 60-fold concentration). This was then filtered into an HPLC vial for storage at 4°C and later analysis.

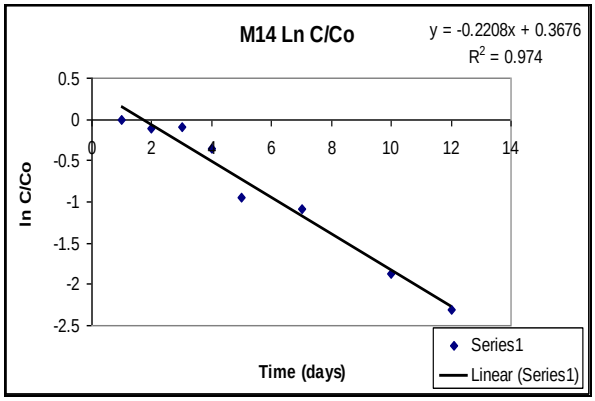
Results and Discussion

Dissipation was characterized using the first-order rate equation similar to that used by Zanella *et al.* (2003) for rice fields. The final derived equation for estimating the half life of dissipation is (where *k* is the dissipation rate constant):

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

The correlation coefficients for all the fields were very high except M2 (Figure 2).





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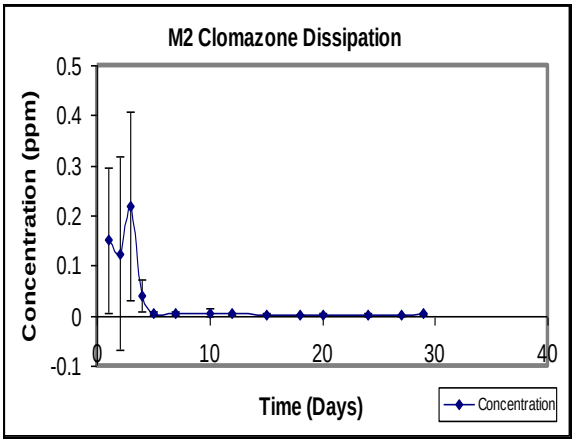

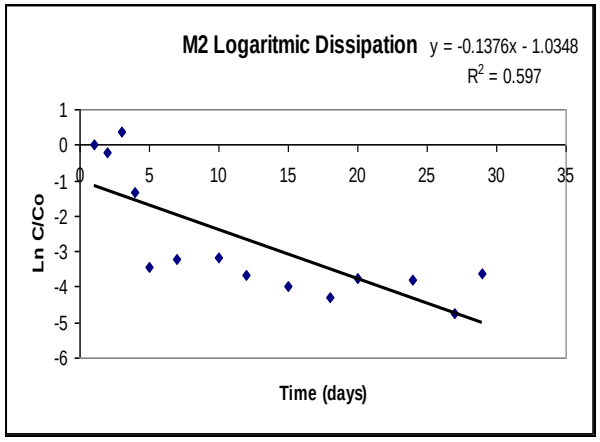

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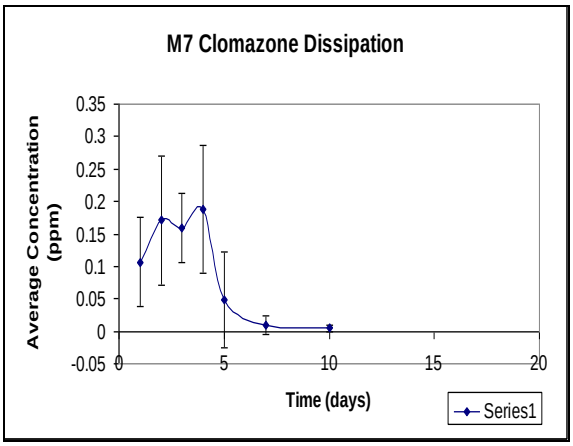

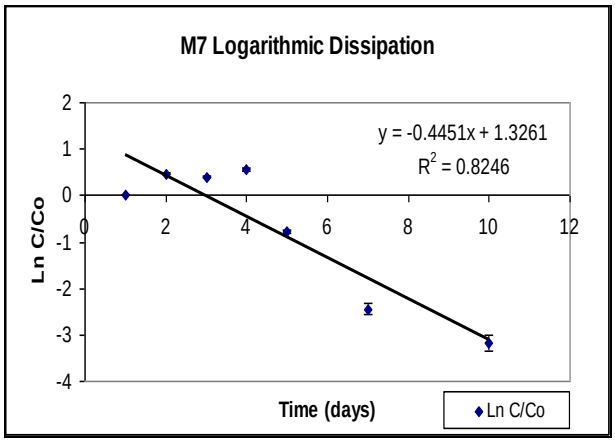

n = 4

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Figure 2. Profiles of Cerano dissipation in the three fields.

Although water samples were taken from the fields over a period of 90 days, Cerano was not detected after day 14. On looking at all the graphs, an initial decline was observed on day1 of the

Table 2. Dissipation summary of clomazome by field.

Site	Chemical	Theoretical Conc. (ppm)	Max Conc. (ppm)	Min. Conc. (ppm)	DT_{50} ($t_{1/2}$)		R^2 Value
M7 North	Cerano	0.44	0.27	0.006	1.63	y = -0.4254x + 0.126	0.811
M7 South	Cerano	0.44	0.14	0.003	1.39	y = -0.4981x - 0.04063	0.7758
M7 Combo	Cerano	0.44	0.19	0.004	1.56	y = -0.4451x + 1.3261	0.8246
M2	Cerano	0.44	0.22	0.001	5.04	y = -0.1376x - 1.0348	0.597
M14	Cerano	0.44	0.06	0.006	3.14	y = -0.2208x + 0.3676	0.974

application of Cerano. This is then to seen to rise again before reaching its peak at day 4, suggesting that Cerano's dissolution in water will depend on temperature and flow rate. Another possible reason may be that the concentration of Cerano at particular sampling points may not be homogeneous. Cerano was sprayed in the air, one would assume that it would be uniform in its distribution in the water but the wind conditions could also play a factor.

The theoretical concentration of Cerano in the rice fields was calculated to be 0.44 mg L^{-1}. This value is dependent on assuming the water depth was an average of 4". The typical range of the ricefield water depth was 2-6". The maximum observed concentration for Cerano in water was 0.27 mg L^{-1} which is 60% the theoretical value. Other possible sources of Cerano dissipation were soil sorption, microbial degradation and photolysis. However, according to the CDPR, Cerano is relatively stable under sunlight hence no significant loss would be expected via photolysis. The CDPR has reported that Cerano degrades slowly under aerobic conditions with half-lives ranging 9-276 days and 60 days on average on anaerobic soils. This shows that some of the Cerano may be sorbed to the soils.

The concentration of Cerano in all the fields was negligible after 14 days. Half-lives were approx. 5 days for M2, 2 days for M7 and 3 days for M13 (Table 2). A 5-day half-life has been observed for Cerano in field water in a field dissipation study as reported by CDPR. Zanella *et al.* (2003), depending upon conditions, also reported half-lives of 2, 3.1, 3.4 and 3.2 days[1]. Most notable is that theoretically within 6 half lives more than 98% of a pesticide would be expected to have dissipated. That appears to be the case in this study, as no detectable concentrations were found after 14 days. The strength of such a field study is that field dissipation is the sum total of

all four contributing fate processes – volatilization, soil sorption, microbial degradation and photolysis. The half lives are within the range of those previously reported from our laboratory studies, confirming the value of using microcosms that model California ricefield conditions. Also, the half lives indicate that Cerano is a relatively safe herbicide for use in California ricefields, as it dissipates rapidly following application and is not likely to end up in drainage water or the Sacramento River.

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Meeting Proceedings

Vasquez, M. E., Gunasekara, A. S., Cahill, T., Tjeerdema, R. S., 2009. Partitioning of etofenprox under simulated California rice growing conditions. *Proceedings of the Northern California Chapter of the Society of Environmental Toxicology & Chemistry*. Davis, CA.

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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 (reversible bonding) to soils, degradation by sunlight and degradation by soil microbes.

2. For the insecticide etofenprox, the degradation rates, half-lives and pathways via both anaerobic and aerobic microbial processes (at 22°C) were characterized. In summary, the insecticide is more efficiently degraded under aerobic, versus anaerobic, conditions (aerobic half life, 27 days; anaerobic half life, 100 days). Similar experiments at 40°C are in progress.

3. For the herbicide Cerano, the degradation rates, half-lives and pathways via both anaerobic and aerobic microbial processes (at 30°C) were also characterized. In summary, and contrary to the results for etofenprox, Cerano is more efficiently degraded under anaerobic, versus aerobic, conditions (aerobic half life, 47 days; anaerobic half life, 8 days).

4. For the herbicide clothianidin, experimental determination of the Henry's law constant to estimate its potential to volatilize was unsuccessful due to its high water solubility and low volatility. The calculated value, though larger than that reported by the manufacturer, is both very low (5.65×10^{-8} versus 2.9×10^{-11} Pa-m^3/mol at 20 °C) and not influenced by increased temperatures. Thus, clothianidin is not likely to dissipate from rice fields via volatilization.

5. For the herbicide Cerano, overall field dissipation was characterized using several ricefields. All concentrations declined to below detection within 14 days, and half-lives ranged approx. 2-5 days, confirming the results of prior laboratory studies. Thus, Cerano appears to be safe for use in California ricefields, as it dissipates efficiently following application and is not likely to end up in drainage water.