

Annual Report Comprehensive Research on Rice

January 1, 1995 – December 31, 1995

Project Title: The Environmental Fate of Pesticides Important to Rice Culture.

Project Leader and Principle UC Investigators:

Project Leader: D.G. Crosby, Environmental Toxicology Dept., UC-Davis.

Principle Investigators:

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Cooperators:

D.E. Bayer (UCD Dept. of Botany); L. Beem (Rhone-Poulenc Ag. Co.); L. Godfrey, UCD Dept. Of Entomology; E. Roncoroni (UCD Dept. of Botany); David Sills (consultant, Sutter Co.); and J.F. Williams (Cooperative Extension, Sutter Co.)

Level of 1995 Funding: \$44,280

Objectives and Experiments Conducted by Location to Accomplish Objectives:

- A. Objective I.** To identify, investigate, and model environmental factors which govern movement and chemical fate of rice pesticides.
 - 1. Environmental Reactivity of carbonate radicals (UCD).
 - 2. Copper mobilization in field soil (UCD).

- B. Objective II.** To estimate the relative importance of such factors to the practical use of specific rice pesticides.
 - 1. Distribution and fate of copper sulfate applied to soil and water (UCD and Sills Ranch, Sutter Co.).
 - 2. Copper toxicity to rice (UCD and Rice Research Station, Biggs).
 - 4. Deschlorothiobencarb generation and degradation (UCD and Placer Co.).
 - 5. Thiobencarb toxicity and inhibition (UCD and Sutter Co).

- C. Objective III.** To apply research results toward meeting regulatory requirements and improved management of rice pesticides.
1. Fipronil persistence and fate in treated fields (UCD and Rice Research Station, Biggs).
 2. Fipronil photodegradation (UCD).
 3. Fipronil analysis by NMR (UCD).

Summary of 1995 Research (Major Accomplishments) By Objective:

A. Objective I.

1. Pesticide Persistence: Carbonate Radicals

Carbonate radicals ($\bullet\text{CO}_3^-$) are highly reactive oxidizing agents that form in field water by the action of sunlight-generated hydroxyl radicals on natural carbonate and bicarbonate ("water hardness"). Our 1994 Report showed that carbonate radicals were detected in rice field water at a steady concentration of about 10^{-14} molar (1 part per trillion) and reacted rapidly with common rice pesticides. This year, a close pesticide relative, *N, N*-dimethylaniline (DMA), was used to test the influence of common water variables on carbonate radical reactivity.

DMA was only moderately degraded by sunlight in distilled water or field water from which carbonate and bicarbonate had been removed (Fig.1). However, addition of increasing levels of the hydroxyl radical generator nitrate to nitrate-free field water resulted in a reduction of the DMA half-life from about 8 hours to less than one hour at the highest nitrate level (25 ppm); bicarbonate at 220 ppm reduced it to 1.3 hours. A normal field water concentration of nitrate is about 10 ppm and that of alkalinity (bicarbonate) about 250 ppm (Sills Ranch), but Central Valley irrigation water occasionally contains over 100 ppm nitrate and 350 ppm bicarbonate. Addition of 176 ppm of an antioxidant, ascorbic acid, returned degradation to the control level, showing that DMA destruction was indeed due to oxidation.

The principal DMA degradation product in irradiated field water in the presence of bicarbonate was *N*-methylaniline, indicating that the main reaction was dealkylation. In the absence of bicarbonate, a completely different product was obtained, and *N*-methylaniline formation was negligible. We conclude that sunlight-generated carbonate radicals are the principal agents responsible for most pesticide photodegradation in rice field water and that breakdown products from it may be different from those obtained by the usual experimental tests used by the manufacturers and by EPA.

2. Copper Mobilization

The possibility that the relatively high levels of insoluble copper detected in some ricefields could become mobilized and bioavailable due to application of common fertilizers or other chemical cannot be discounted. However, laboratory tests this year produced ambiguous results. It seems clear that further work will be required.

B. Objective II.**1. Copper in Ricefield Water**

Last year's Report concluded that very little of the copper sulfate ("Bluestone") applied commercially to a rice field remained in the water column. However, the continued concern of State regulatory agencies over the possibility of toxic levels of copper in released tailwater prompted further investigation. Copper sulfate pentahydrate was applied at a nominal 15 lbs/acre (16.8 kg/ha) by aircraft to three commercial rice fields in Sutter County: Field # 1, 52 acres (21 ha), off Sankey Rd. West of Hwy 99; Field #2, 108 acres (43.7 ha), off Hwy 99 halfway between Sankey Rd. and Howsley Rd.; and Field #3, 30 acres (12.2 ha), off Howsley Rd. East of Hwy 99. Duplicate water samples were taken at intervals at the outlet of each field, composited, and analyzed for total Cu. The results (Table I) were very similar to those of 1994; combined particulate and dissolved copper never exceeded 0.4 ppm in tailwater as it left the field and declined with time from a calculated high of 17 ppm (assuming all the bluestone dissolved).

Table I. Total Copper in Ricefield Tailwater

<i>Field</i>	<i>Analytical Method</i>	<i>Sample Time Hours postapplication</i>	<i>pH</i>	<i>Total Cu, ppm</i>
1	ICP (DANR Lab)	24	7.8	0.06
		72	7.4	0.10
2	ICP (DANR Lab)	7	8.2	0.40
		48	7.9	0.21
3	Bathocuproine	50	8.6	0.01
		98	-	0.00

The Federal Water Quality Standard for copper is 1.00 ppm for drinking water and one-tenth the 96-hour median lethal concentration for protection of aquatic animals (Table II). The average copper concentration in over 1100 surface water samples in the United States (Kopp and Krone, 1967) was 0.015 ppm (15 ppb), with a maximum of 280 ppm (280 ppb).

Except for the 7-hour sample, ricefield water contained 100 ppb or less of total copper, and the level would be expected to decline with time and with movement of the water. Still, at the required 0.1 LC₅₀, these levels by themselves often may not meet the federal standards presently used also by the State. However, if part of the copper were in particulate form, some type of simple filtration of tailwater or a holding period might sufficiently reduce the levels leaving the fields.

Table II. Copper Toxicity to Representative Aquatic Species

Name	Species	Size	Temp., °C	pH	96-hr LC ₅₀ , ppb
Chinook Salmon	<i>O. tshawytscha</i>	hatchling	11-12	7.0	31
Bluegill sunfish	<i>L. macrochirus</i>	1-2 g	25	7.5	19.5
Striped bass	<i>M. saxatilis</i>	2.7 g	21	8.2	620
Carp	<i>C. carpio</i>	adult	28	8.0	800
Mosquito fish	<i>G. affinis</i>	adult	24-27	6.1 - 8.1	75,000
Water flea	<i>D. magna</i>	adult	25	?	44 (3 week)

2. Toxicity of Soil Copper to Rice Plants

With the cooperation of D.E. Bayer and E. Roncoroni, rice plants were grown in the greenhouse in flooded soil containing copper at the natural background level (45 ppm Cu) and at a total of 100, 150, 200, and 300 ppm obtained by incorporating appropriate amounts of freshly precipitated basic copper carbonate (malachite) before planting. The seeds appeared to germinate normally, and the 100 ppm plants appeared even taller and greener than controls. However, plants at other concentrations were increasingly stunted as copper concentrations increased.

Field toxicity tests were conducted in 8 ft (2.4 m) diameter aluminum rings set into soil at the Biggs Rice Research Station. Before placing each ring, the soil inside was rototilled, turned over with shovels, and thoroughly mixed with a slurry of freshly precipitated basic copper carbonate prepared on-site. This is the same form of copper that precipitates when field water is treated with bluestone. The plots and rings were flooded, seeded, and otherwise treated according to commercial practice. The rice was harvested and threshed mechanically in late October.

Results are shown in Table 3. Control plants appeared normal, and although well below average, the rice yield equivalent of 3.40 tons/acre was highest. Plants exposed 100 ppm of copper average only 2.57 tons/acre, although the 3.17 tons/acre value of ring 8 could be the better value. At 200 ppm of copper, yield fell to 2.84 tons/acre, or about 84% of controls, and plants exposed to 300 ppm of Cu were stunted and the combined rice yield was only 1.54 tons/acre (45% of controls).

An annual application of 15 lbs/acre of bluestone (29.5%) actual Cu would result in an additional 1.1 ppm of copper in the soil each year (our 1994 estimate of 3.0 ppm was erroneous). At this rate, at least 100 years will be required for serious and permanent yield reduction to appear in most of the California rice fields tested so far. Nonetheless, as some alternate crops might be

more sensitive, and so might new varieties of rice, prudence suggests that a search for a more satisfactory algicide be initiated.

Table III. Effects of Soil Copper on Rice

<i>Ring</i>	<i>Appearance of Crop</i>	<i>Rice Yield g.</i>	<i>Rice Yield Tons/Acre</i>
5	Normal	2776	2.73
7	Normal	4136	4.07
3	Normal	2015	1.98
8	Normal	3212	3.16
4	Normal?	3219	3.17
6	Normal?	2560	2.52
1	Stunted	1092	1.08
2	Stunted	2035	2.00

3. Deschlorothiobencarb Generation, Degradation, and Toxicity

Soil samples were collected in 1994 from thiobencarb-treated rice fields off Brewer Road, where stunting of the crop was clearly visible. They represented the center of a stunted area (A), its periphery (B), and an adjacent healthy area (C) of the same field. Analysis related stunting to the level of Deschlorothiobencarb (DCT) derived by dechlorination of the thiobencarb (TB). Addition of 5 ppm of TB to the most active soil (A) under waterlogged (anaerobic) conditions showed that DCT was generated from the herbicide (Fig. 2, from in the 1994 Report).

Dechlorination stopped when the soil was dried and became aerobic. When Soil A was steam-sterilized, the level of added TB level remained unchanged over a period of 60 days, and DCT likewise remained at a constant low level. When control soils were fortified with 5.15 ppm of DCT and held in the laboratory up to 70 days under either aerobic or anaerobic conditions, the DCT level slowly dropped to 4.6 ppm during 20 days in the anaerobic soil, while that in the aerobic soil quickly fell to 2.5 ppm. At day 70, the values were 3.8 and 1.9 ppm, respectively. It is apparent that DCT is produced by the action of anaerobic microorganisms on TB.

But is DCT toxic to California rice? Pure DCT was synthesized in the laboratory and tested on greenhouse rice with the help of D.E. Bayer. Soil was fortified with TB and DCT at 0.0, 0.5, 1.0, 2.0, and 4.0 ppm, similar to the levels found in TB-treated fields. At maturity, plants were harvested, cleaned, measured, and weighed. The results (Fig. 3) clearly show that DCT causes stunting of rice

in proportion to its concentration; TB-treated plants were all healthy and closely similar to the controls in length and weight.

Can anything be done to correct this situation? One possibility, perhaps impractical, would be to allow soils to dry out as soon as damage was detected. Another would be to control the microorganisms responsible. Toxic soil was collected from the same areas as before, fortified with 5 ppm of TB, incubated with either the fungicide benomyl (10 mg/kg) or the antibiotic streptomycin (3g/kg), and both TB and DCT measured at intervals. As shown in Fig. 4, benomyl had no effect at this level, but streptomycin effectively inhibited the degradation of TB and the formation of DCT.

C. Objective III.

1. Fipronil persistence and fate

Fipronil is a new insecticide discovered by Rhone-Poulenc Ag Co. and found by Dr. L. Godfrey to be highly effective against rice water weevils. It shows relatively low toxicity toward mammals, birds, fish, and aquatic invertebrates and is effective at very low application rates, typically 11 grams of active ingredient per acre (0.025 lbs/acre). Its field dissipation has not been determined previously.

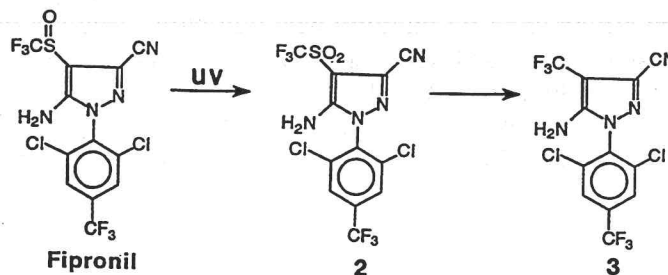
First, it became necessary to develop an analytical method that would detect and measure Fipronil and its expected degradation products at the exceptionally low levels of less than the calculated maximum of 28 $\mu\text{g/L}$ (28 ppb) in water and 12 $\mu\text{g/kg}$ (12 ppb) of soil. As this had to be done long before the rice season, recoveries were made from distilled water or Putah Creek water, and from rice soil that had been held in frozen storage. Gas chromatography of extracts with an N-P detector eventually provided 83-99% recovery of standards and a sensitivity of <1 ppb.

Experimental plots of 10 x 20 ft were established within metal rectangles by Dr. Godfrey at the Rice Research Station, and treated with several Fipronil formulations at 0.025 lbs ai/acre: Plots #3 contained granules soil-incorporated before flooding, #4 contained dry flowable soil-incorporated before flooding, #5 plots received granules distributed into the water at the 3-leaf stage, and RP plots were similar to #4 but were treated by Dr. Lance Beem of Phone-Poulenc.

Water and soil from treated and control plots were sampled in duplicate as soon as possible after flooding: 24 hours for #3 and #4, 15 minutes for #5, and 12 hours for RP plots. Samples were removed from 68 locations within each enclosure, composited, and returned chilled to UCD for analysis. The dissipation curve for water-applied Fipronil (#5-1) is shown in 5A, and that for #5-2 was very similar. After an initial period of dispersion from the granules, in which the aqueous concentration rose rapidly to a maximum of 6 ppb, the Fipronil dissipated according to first-order kinetics with a half-life of 67 hours. At 411 hours (17.1 days), the Fipronil level was 500 parts per trillion, and sampling was discontinued. Fig 5B shows a similar result for the RP plot (soil incorporation) and a half-life of 63 hours in the water. Curiously, no Fipronil was ever detected in the water of plots #3 or #4 down to a detection limit of <1 ppb. Analysis of soil residues, not completed yet, may help to explain this.

A Fipronil degradation product appeared sporadically in plot #5 near the limit of detection but was clearly present in RP water (Fig. 5B); it was determined to be Compound 3. While the

parent compound dissipated to the limit of analytical detection within about 150 hours, Compound 3 increased and then only slowly dissipated. At about 300 hours, the water level in the field dropped, resulting in anomalous analytical results.



A series of laboratory dissipation experiments was conducted on Fipronil in sunlight in field water alone, field water over a layer of soil, and in distilled water, but at higher concentrations. The results were very similar to those from the field trials and to each other (Fig. 6). However, in this case, the photodegradation product, Compound 3, did not dissipate up to 200 hours. The containers were closed, so volatility was not a factor for Fipronil but could explain the seeming stability of Compound 3. The similarity of Fipronil dissipation in all three experiments showed that neither natural oxidants nor soil were important to the degradation.

At these very low Fipronil levels, no other degradation products were detectable by gas chromatography, so the fluorine NMR analytical method described in our 1994 Report was applied to Fipronil solutions exposed to sunlight. The NMR spectrum from the photodegradation in aqueous methanol revealed Fipronil, Compound 3, and the otherwise undetected intermediate compound 2 to be present from both the irradiation in aqueous methanol and from extracts of field water during the dissipation experiment.

Publications and Reports

1. Mabury, S.A., and D.G. Crosby. 1995. ^{19}F -NMR as an analytical tool for fluorinated agrochemical research. *J. Agr. Food Chem.* 48:1845-1848.
2. Crosby, D.G. 1995. Persistence of pesticide residues: Transport, fate, effects. *Annual Report of USDA Regional Research Project W-45*, Corvallis, OR.

Concise General Summary of Current Year's Results

1. Sunlight-generated hydroxyl radicals have long been thought to be the most important reactants for degradation of ricefield pesticides. We now believe that the carbonate radicals formed in water by reaction of hydroxyl and natural bicarbonate ("hardness") may be of equal or greater importance, although the standard degradation tests conducted by EPA and pesticide manufacturers seldom are carried out under conditions where these radicals would exist.
2. Despite most of the copper being quickly precipitated out, the tailwater leaving bluestone-treated fields still contains up to 400 ppb of total Cu. This amount exceeds the Federal-State water quality standards for protection of aquatic animals, but the level drops over time and distance traveled.

3. In both the greenhouse and field, soil levels of copper above 100 ppm cause stunting of rice plants and yield reduction. The present level averages about 50 ppm. Although as much as 100 years will be required for copper levels to reach 150 ppm at present bluestone application rates, new rice varieties and alternative crops could be affected in the future, and reduction in copper applications are suggested.
4. Soils from fields treated with the herbicide thiobencarb (Bolero) can cause stunting and toxicity in rice due to formation of dechlorinated thiobencarb (DCT). Synthetic DCT was shown to be toxic to rice plants at the levels found in injured fields. DCT is formed from thiobencarb by anaerobic bacteria and can be removed or prevented by drying the soil in air or by application of the antibiotic streptomycin. This may become especially important with winter flooding of many fields.
5. An analytical method was developed that detects and measures the new insecticide, Fipronil, in ricefield soil and water at levels down to less than 1 part per billion. Using this, the dissipation of Fipronil from field plots was followed. A standard application of 0.025 lbs/acre resulted in 4-6 ppb in field water soon after, declining to 0.5 ppb at 400 hours.
6. The principal route of Fipronil loss was photodegradation in sunlight, with formation of a more persistent desulfurized product. The toxicity of this chemical, which reached a maximum of 2 ppb, might have to be considered. Otherwise, the properties of Fipronil look very promising.

Donald G. Crosby
Project Leader

7 December 1995
date

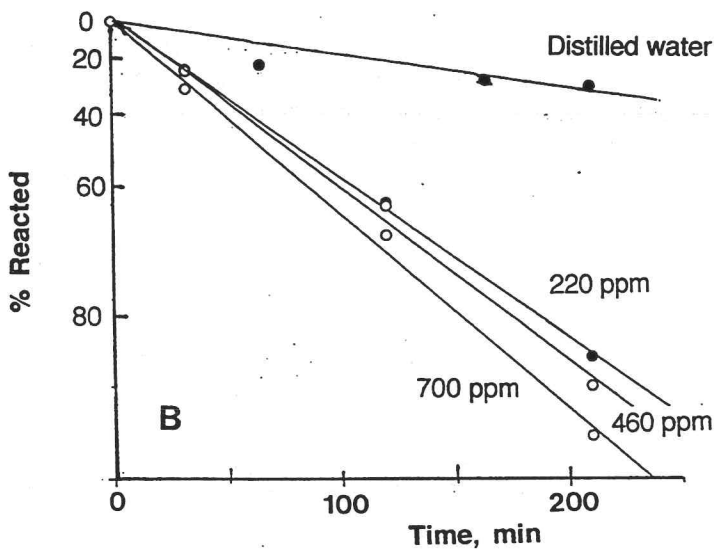
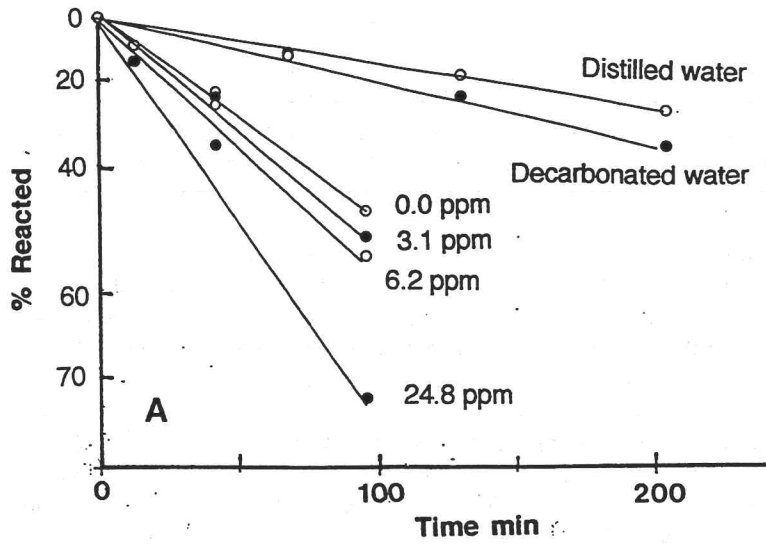


Fig. 1. Degradation of *N,N*-dimethylaniline at various levels of nitrate (A) and bicarbonate (B) added to field water or distilled water.

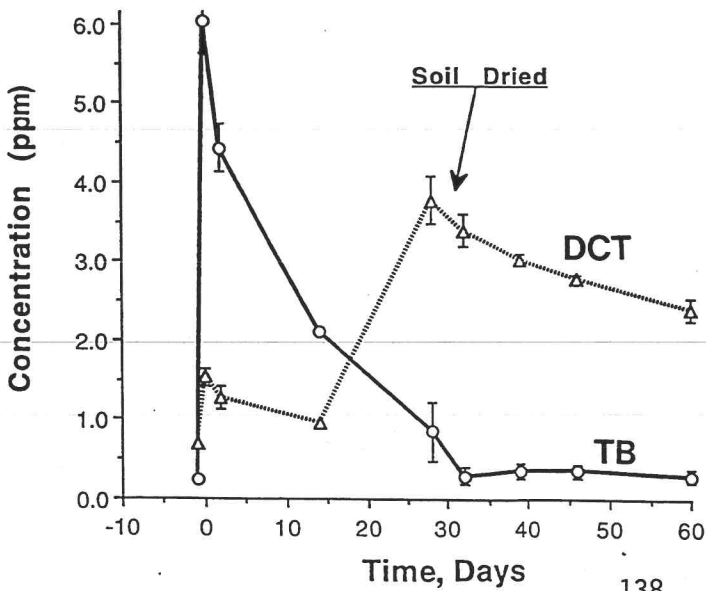


Fig. 2. Conversion of thiobencarb (TB) to deschlorothiobencarb (DCT) in anaerobic field soil.

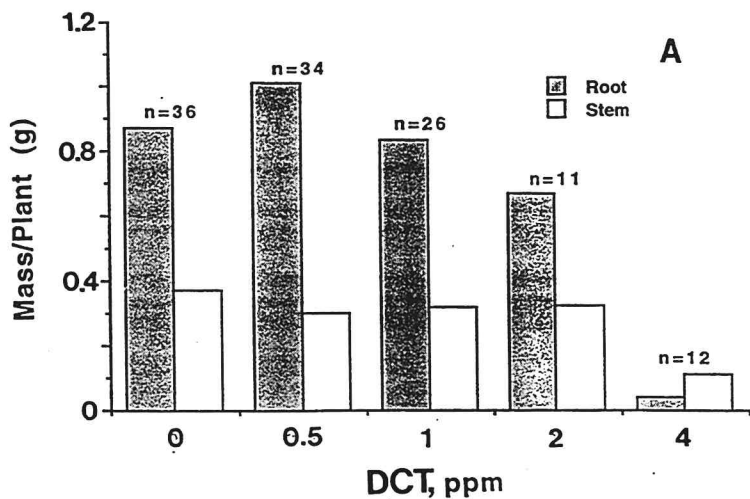


Fig. 3 Effect of DCT on (B) stem length and (A) weight of rice plants.

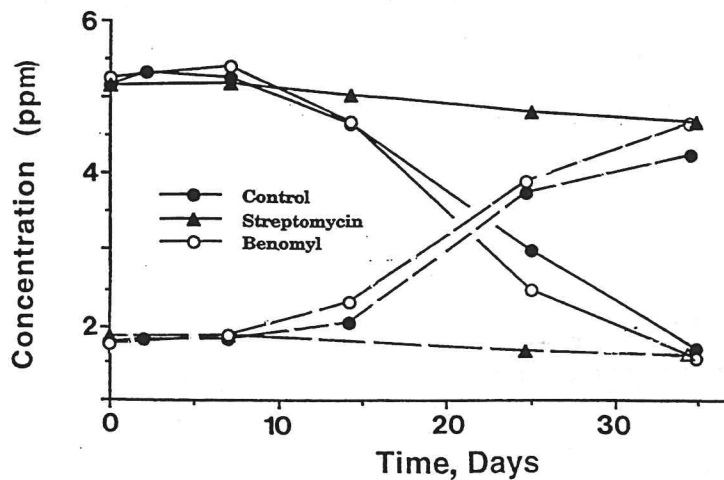
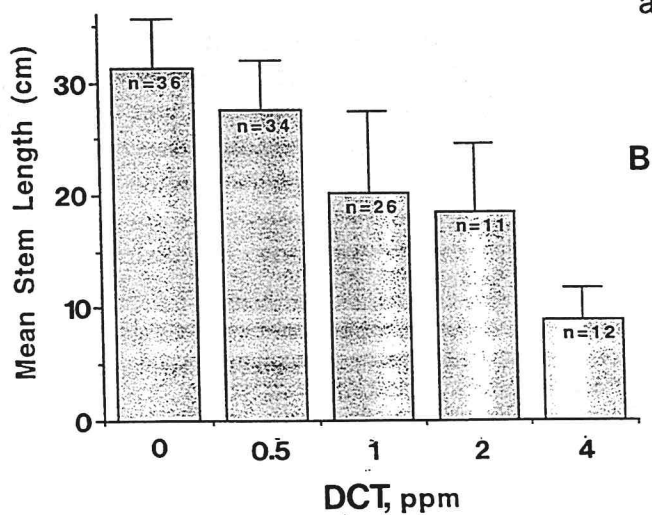


Fig. 4. Effect of benomyl and streptomycin on TB degradation (solid lines) and DCT formation (dashed lines).

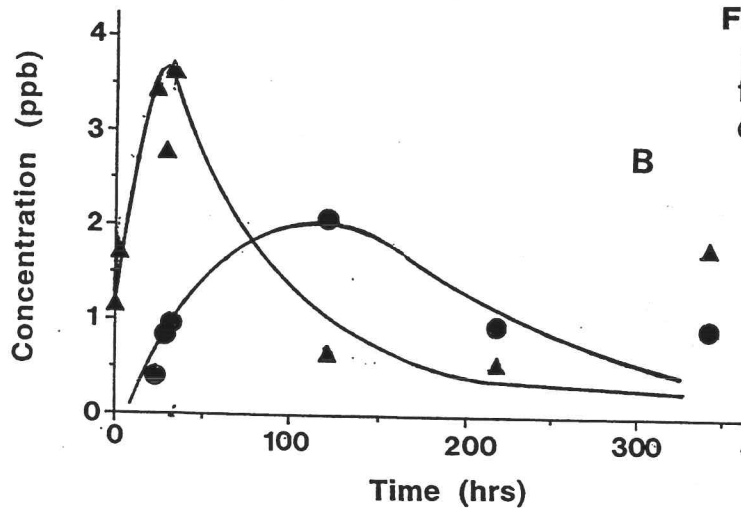
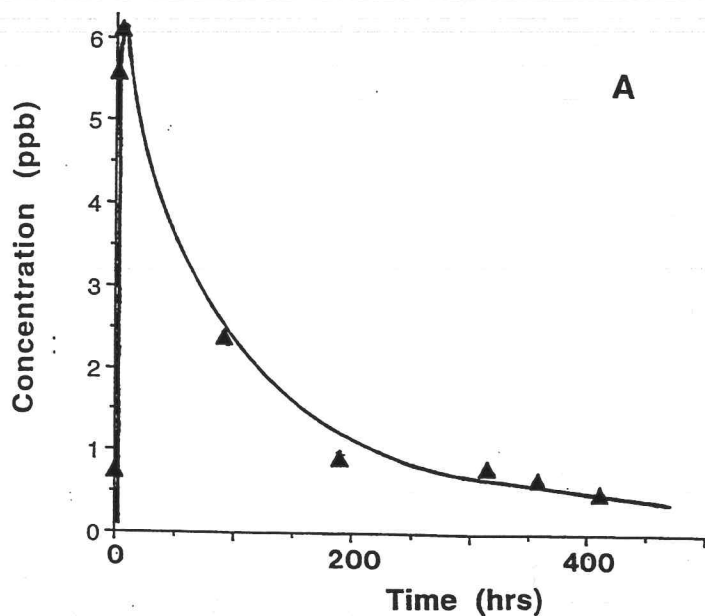


Fig. 5. Dissipation rate of (A) water-applied and (B) soil-incorporated Fipronil from ricefield water (\blacktriangle), and formation of Compound 3 (\bullet).

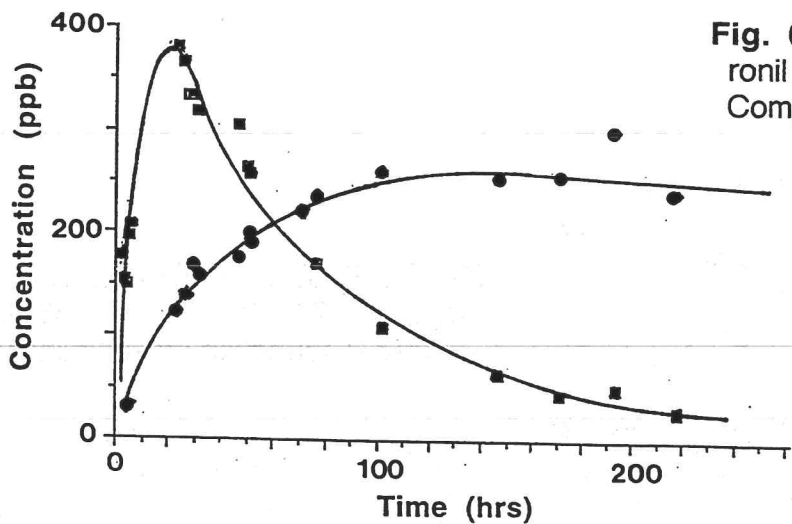


Fig. 6. *In vitro* photodegradation of Fipronil in field water (\blacksquare), and formation of Compound 3 (\bullet).