

Comprehensive Research on Rice

Annual Report

January 1, 1993 – December 31, 1993

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

Project Leader and Principle UC Investigators:

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Level of 1993 Funding: \$46,765

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. Pesticide persistence: hydroxyl radical measurement in natural water (UC-Davis).
 2. Hydroxyl radical generation in field water (UC-Davis; Lake Tahoe; Clear Lake; East Nicholas, Sutter Co.).
 3. Concentration and degradation of pesticides in surface microlayers (UC-Davis; UCD Rice Research Facility; Demeter Ranch, Sutter Co.).

B. Objective II. To estimate the relative importance of such factors to the practical use of specific rice pesticides.

1. Chemical form and analysis of ricefield copper (UCD; 11 sites in Sutter Co.).
2. Field distribution of applied copper sulfate (UCD; East Nicholas, Sutter Co.).
3. Biodegradation of thiobencarb in a ricefield soil (UC-Davis; Merkle Site, Placer Co.).

C. Objective III. To apply research results toward meeting regulatory requirements and improved management of rice pesticides.

1. Comparative movement of pesticides through ricefield soils (UC-Davis).
2. Specific analysis of fluorinated pesticides in soil and water (UC-Davis).

Summary of 1993 Research (Major Accomplishments) By Objective:

A. Objective I.

1. Pesticide Persistence: Hydroxyl Radicals in Natural Waters

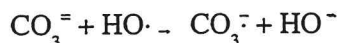
We have established a direct relationship between the field degradation rate of a rice pesticide — a reflection of its persistence — and its reactivity toward hydroxyl radicals in the water. All major sources of hydroxyl ($\text{HO}\cdot$) were shown to be present in rice fields, including hydrogen peroxide, nitrate, nitrite, copper, and iron; our next step was to analyze field water (and other natural water) directly for hydroxyl and also for some of its principal sources.

Hydroxyl was measured by its reaction with methanol added to the water sample followed by quantitative determination of the resulting formaldehyde by high-pressure liquid chromatography (HPLC) of its dinitrophenylhydrazone; copper (Cu) and iron (Fe) were measured colorimetrically with bathocuproine and ferrozine, respectively; hydrogen peroxide was monitored via its generation of a fluorescent pigment from p-hydroxyphenylacetic acid; and nitrate (NO_3^-) was measured by ion chromatography and nitrate electrode. Carbonate radicle ($\text{CO}_3\cdot^-$) was determined with *N,N*-dimethylaniline. Rice field water was fortified with Fe, Cu, Mn, and nitrate at levels existing under typical field conditions in order to determine the mechanism by which these constituents generate hydroxyl as well as the relative importance of each. Water also was collected from Lake Tahoe and Clear Lake to compare absolute concentrations of hydroxyl, carbonate radical, and hydrogen peroxide. Solutions were irradiated for 4 hrs under full summer sunlight.

The production of hydroxyl varied significantly among the different concentrations of metals and nitrate (Fig. 1). Mn was included because it theoretically could be a source of hydroxyl, but no $\text{HO}\cdot$ increase was observed. Fe, Cu, and nitrate provided an increase in $\cdot\text{OH}$ over field water devoid of these constituents. The relative rates of $\cdot\text{OH}$ generation at constant concentration were 3.42 (at $30\ \mu\text{M}$) for Fe, 0.84 (at $30\ \mu\text{M}$) for Cu, and 0.29 (at $400\ \mu\text{M}$) for nitrate. Although nitrate generated hydroxyl at the slowest rate, the high levels of nitrate in many rice fields could make it the major source of hydroxyl. Mechanistically, the generation of hydroxyl differed between Cu and Fe; irradiation of field water fortified with Cu showed a decrease in hydrogen peroxide, while Fe and Mn showed either a small decrease or no increase (Fig. 2). These results indicate that copper is being photo-reduced from the usual Cu (II) state

to Cu (I) that is then able to reduce hydrogen peroxide to hydroxyl. Iron did not show this decrease in hydrogen peroxide, indicating that direct photolysis of aquated iron (FeOH^{2+}) was the most likely source of hydroxyl.

Carbonate and bicarbonate are ubiquitous constituents of natural waters. They "scavenge" hydroxyl radicals by a rapid reaction that generates carbonate radicals and hydroxide ions:



Concentrations of hydroxyl and carbonate radicals differed among the three waters tested (Fig. 3). Clear Lake water is highly alkaline (high carbonate, Table I), resulting in rapid scavenging and low $\text{HO}\cdot$; conversely, the clean Lake Tahoe water has low alkalinity and so a relatively high $\text{HO}\cdot$ production with relatively little scavenging. However, as Table I suggests, carbonate radical concentrations typically are 100–10,000 times greater than those of hydroxyl; although little is known about pesticide reactions with $\text{CO}_3^{\cdot -}$, the sparse literature indicates that many sulfur-containing chemicals such as molinate, thiobencarb, and methyl parathion could react. While hydroxyl remains the most important rice field oxidant, carbonate radical may play a more important role than had previously been suspected.

Table I. Hydroxyl and carbonate radicals in water.

Measure	Clear Lake	Lake Tahoe	Rice Field
$\text{HO}\cdot$ Production (moles/sec)	4.6×10^{-11}	1.3×10^{-11}	6.4×10^{-11}
$\text{HO}\cdot$ Scavenging rate (per sec)	4.9×10^7	7.3×10^5	2.0×10^6
$\text{HO}\cdot$ Concentration (molar)	1.8×10^{-18}	3.0×10^{-17}	1.2×10^{-16}
Alkalinity (mg CaCO_3/L)	191	64	70
Carbonate Radical Conc. (molar)	4.3×10^{-14}	3.1×10^{-14}	1.0×10^{-14}

2. Pesticides in the Surface Microlayer

Most bodies of water, including flooded rice fields, are at least partially covered by a very thin film ("microlayer") of natural organic material derived from decaying plants and animals. Many methods have been reported for collecting microlayer from the surface of both freshwater ponds and the sea, but their efficiency is low and the microlayer is greatly diluted with entrained water. We have developed a very simple and efficient collection method based on immersing and withdrawing a glass plate which has been rendered water-repellant by bonding a silicone to its surface; the sticky microlayer is subsequently rinsed off into a bottle by a stream of organic solvent (usually ethyl acetate). The microlayer is very thin, weighing only about 500 mg per m^2 of water surface.

Typical rice field microlayers were analyzed and appear to be composed largely (~96%) of protein and carbohydrate, with the remaining percentage representing mostly C_8 – C_{20} fatty acids and their methyl esters. Cholesterol and the C_{26} and C_{28} fatty acid methyl esters were identified but not quantitated, and the microlayer's green color indicated substantial chlorophyll. The composition suggests that the lipid comes primarily from algae.

Pesticides can concentrate in the microlayer. Field plots (4.27 x 2.29 m) at the UCD Rice Research Facility were sprayed with carbofuran (Furadan® 40EC) at a normal application rate of 3.27 kg/ha (2.92 lbs/acre), and the microlayer was sampled at intervals and analyzed. Figure 4 shows that while the "wet" microlayer sampled by the usual (silanized) plate method contained somewhere near the same concentration of pesticide as did the underlying water (< 50 ppb except immediately after spraying), the true concentration in the microlayer, as measured with silanized plates, was closer to 10^7 ppb (1% by weight). These concentrations declined with time (Fig. 4a), presumably due to carbofuran degradation and partitioning into the subsurface water. Thiobencarb, applied as an 85% EC formulation of Bolero at 3.41 kg/ha (3.04 lbs/acre), behaved similarly (Fig. 4b). Sampling of a commercially-treated rice field showed that the microlayer contained 10,000 times higher concentration of carbofuran and 100,000 times higher concentration of thiobencarb than appeared in the underlying water.

In a laboratory experiment, pure thiobencarb was dissolved in a known weight of collected microlayer (3.6 ng/cm^2 , or approximately $70 \text{ } \mu\text{g/g}$ of microlayer), deposited on the inner wall of glass test tubes and irradiated in a solar-simulating photoreactor. Controls included dark conditions (photoreactor turned off) and thiobencarb film without microlayer. As shown in Fig. 5, a little thiobencarb degradation occurred even in the dark, perhaps due to microbial action, but extensive degradation was observed under ultraviolet irradiation. The only photodegradation product detected was p-chlorobenzyl mercaptan, and none of the expected dechlorinated thiobencarb was found.

These experiments show that (1) the surface microlayer concentrates hydrophobic pesticides to an unexpectedly high degree, (2) the pesticides may dissipate more slowly from the microlayer than from subsurface water, and (3) presence of high levels of pesticide residue in the microlayer can be expected to influence the accuracy and precision of water analyses.

B. Objective II.

1. Copper in California Rice Fields

Copper sulfate pentahydrate (bluestone) is applied in large amounts in California rice culture for the control of algae and tadpole shrimp. The 1991 Pesticide Use Report showed that just over 1.5 million pounds were applied that year to 97,000 acres (15 pounds/acre), which represents over 375,000 pounds of elemental copper. Our objectives were to determine where all this copper goes; how much leaves the field in tailwater, is added to the soil, or drifts off-site; and how fast it is dissipated from the water. Additionally, we sampled soils throughout Sutter County to evaluate the present soil burden of copper from previous applications and provided fortified soil samples to Dr. Bayer to assess the relationship between copper load in soil and injury to rice seedlings.

Copper (Cu) in water was analyzed spectrometrically after derivatization with bathocuproine, or by ICP spectrometry at the DANR laboratory at UCD. Soil samples were oven-dried, ground, sieved, and analyzed for total and bioavailable Cu. Field experiments were carried out on a 12 acre rice field northeast of East Nicholas; soil, water, and air deposition samples were collected, and dishes filled with soil were submerged in the field. Five separate water sampling sites included one at the outlet, and beakers were placed in the field along two transects. Samples were taken at appropriate intervals for 4 days following aerial application of copper sulfate pentahydrate at a nominal rate of 15 pounds/acre (16.8 kg/ha).

Dissolved copper in the field water averaged 2.1 ± 0.7 ppm at 10 min post-spray and declined rapidly to 0.21 ± 0.08 ppm within 12 hrs (Fig. 6). The final sample (79 hrs) averaged 33.9 ± 27.4 ppb Cu

throughout the field. At 2 hrs post-application, the tailwater concentration was similar to that of the rest of the field (maximum of 1.3 ppm). Soil averaged 54 ppm of total Cu and 10 ppm bioavailable Cu before application, which increased significantly (average 16 ppb) post-application (Fig. 7), although no general trend was observed during the rest of the sampling; once in the soil, the Cu remained fixed. Soil residues at 10 sampling times and 2 locations averaged 69 ppm total Cu and 17 ppm bioavailable Cu, an increase in the top 1 cm of soil of 28% in total Cu and 70% in bioavailable Cu. Copper dissipated only slowly from water when no soil was present, but adding soil resulted in an almost instantaneous decrease which reached over 90% within 5 hrs.

Although the wind was light on the day of application, we observed detectable CuSO_4 residues in the beakers out to 100 m; there was a general decline from 3.5 mg of Cu per m^2 of soil surface at 5 m from the water to undetectable at 172 m. While there was significant variability between the two sampling transects, these results indicate that copper may be transported off-site by drift.

Within 10 min after copper sulfate application, 72% of Cu could be accounted for in the field water, <1% on adjacent soil, and a negligible amount in air. However, after 6 hrs, 88% of the applied Cu was found in the top centimeter of submerged bottom sediment, precipitated as mineral malachite (copper hydroxycarbonate) pending final identification. Loss via spilled tailwater was not measured due to sampling difficulty but was estimated to be minor. As algae are killed primarily at aqueous concentrations above about 1000 ppb, very little time was available for the copper to become effective (Fig. 6).

Soil core samples collected at 10 other rice field locations in Sutter county showed total Cu to be between 15 and 54 ppm (3.9 to 10.4 ppm bioavailable Cu). Soils containing the higher total Cu levels generally had correspondingly high bioavailable Cu, but not always. At 50 ppm, these soils presently would contain approximately 160 lbs of copper per acre. The rate of copper sulfate application can only be presumed to have been correct. After time-consuming preparation for our first field experiment and movement of the aircraft over the field, it was found that almost no copper sulfate had actually been applied. This suggests the possible need for a more formalized process of spot-checking aerial applications in the future.

2. Biodegradation of Thiobencarb in a Rice Field Soil

Although Japanese scientists in 1980 had associated severe stunting of field rice with the presence of a thiobencarb (TB) degradation product, deschlorothiobencarb (DTB), generated by soil bacteria (*Pseudomonas* species), our 1992 Report described laboratory experiments which failed to demonstrate the formation of this chemical in four typical California rice field soils. However, the increasing observation of similar stunting in parts of thiobencarb-treated Central Valley fields led us to analyze soil samples (San Joaquin Sandy loam) from one such area (Merkle Site near Catlett Road in Placer County).

Samples were collected from a stunted check and an adjacent normal (control) check in August, 1993; they were extracted repeatedly with acetone and the extracts analyzed by capillary gas chromatography. The analysis clearly distinguished between TB and authentic DTB synthesized in our laboratory. Although standard recoveries were low, Table II shows that soil from the stunted area contained a (corrected) average of 3.4 ppm of thiobencarb even three months after application, and also a relatively high 7.2 ppm of the toxic DTB whose identity was confirmed by mass spectrometry. Rice seedlings grown in this soil by Dr. Bayer showed typical injury, while those grown in the control soil did not, although the control soil also contained detectable TB and DTB (0.4 ppm and 0.1 ppm, respectively).

Table II. TB and DTB Concentrations in soil (ppm).^a

Replicate	Normal Rice		Stunted Rice	
	TB	DTB	TB	DTB
1	0.4	0.126	2.954	5.832
2	0.329	0.119	3.59	8.2
3	0.382	0.126	3.919	9.266
4	0.436	0.148	2.958	5.51
Average	0.387	0.131	3.354	7.202
Std. Dev.	0.046	0.012	0.481	1.824
% Recovery	28	42	52	41

^aConcentrations corrected for % recovery.

These findings strongly implicate thiobencarb dechlorination as the cause of the injury, indicate that the factors necessary for the anaerobic degradation of thiobencarb now exist in the Central Valley, and suggest that the relation of injury to straw incorporation and continuous flood—both of which may increase dechlorination—should be investigated.

C. Objective III.

1. Comparative Movement of Pesticides through Rice Field Soils

Following our observation and measurement last year of the unexpected *lateral* movement of quinclorac and KI-2023 through even heavy clay soils, we proposed to develop a quick and simple index of potential mobility to predict the movement of pesticides—existing and future—through California rice soils. Ideally, the methods would not require radioactive labelling in test pesticides, which seriously complicates both their scope and utility. Our efforts to visualize the location of pesticides on soil chromatoplates and columns with fluorescent dyes have been unsuccessful so far, but work is continuing.

2. Specific Analysis of Fluorinated Pesticides in Soil and Water

Two major difficulties in the analysis of pesticides and their degradation products in soil and water have been the quantitative separation of the complex mixture of analytes, and the interferences caused by the numerous natural and manmade chemicals present in the samples. Both of these problems might be solved by a detection method that was specific only for the analytes of interest. For fluorinated pesticides, such as the experimental rice pesticide diflubenzuron, fluorine-specific nuclear magnetic resonance spectrometry (¹⁹F-NMR), might serve.

This technique measures *only* fluorinated compounds, specifically, and the NMR signal for each substance is unique and so allows identification. In previous Reports, we have described the degradation of the insecticide diflubenzuron (DFB), Dimilin®, in tailwater to give 2,6-difluorobenzoic acid (DFBA). While DFBA proved difficult to extract quantitatively at low levels in field water, its characteristic NMR

signal was easy to detect and measure in degradate mixtures (Fig. 8). Another example, developed as a model, is the fluorinated herbicide, trifluralin; aqueous trifluralin (Fig. 9) was exposed to sunlight and the sample tube periodically placed in the NMR spectrometer to monitor the trifluralin photodegradation into two major products, mono- and didealkylated trifluralin, readily distinguished by their NMR spectra. After 120 min, no trifluralin remained. No extractions, interferences, or separations were involved.

Although this method will be used to follow further the fate of diflubenzuron in rice field water, where all other methods have proved unsatisfactory, it also holds future promise for investigation and monitoring of other fluorinated compounds which now represent 12% of all new pesticides.

Publications and Reports

J.R. Gever. *Rice Field Surface Microlayer: Collection, Composition, and Pesticide Enrichment*. M.S. Thesis, UC-Davis, September, 1993, 63 pp.

S.A. Mabury and D.G. Crosby. The photo-oxidative capacity of natural waters, *Abstr 14th National SETAC Meeting*, Houston, TX, Nov. 1993, No. P823.

S.A. Mabury and D.G. Crosby. ^{19}F NMR as an analytical tool for monitoring fluorinated pesticides. *Abstr. 205th National Meeting, Amer. Chem. Soc.*, Denver, CO, March 1993.

S.A. Mabury. Hydroxyl radical in natural waters. Agricultural and Environmental Chemistry Seminar, UC-Davis, October 18, 1993.

Concise General Summary of Current Year's Results

1. While dissolved iron, copper, and hydrogen peroxide all contribute to the natural capacity of rice field water to degrade pesticides, nitrate (largely from fertilizer) is generally the most important source of the degradative agent, hydroxyl radical. Another significant but previously unrecognized agent is the carbonate radical generated from hydroxyl and dissolved carbonate minerals ("hardness").
2. The dechlorinated metabolite (DTB) of thiobencarb (TB) is implicated in the occasional stunting of California rice observed during the past several years. Soil in a normal field contained 0.4 ppm of TB and 0.1 ppm of DTB, while soil in which rice plants were injured contained 3.4 ppm of TB and 7.2 ppm of DTB. This indicates that the factors necessary for DTB formation already exist in Central Valley rice soils.
3. Copper sulfate (bluestone) is heavily used for pest control in California rice. Although small proportions of aerially-applied copper were found in adjacent air, soil, and water, 88% of the application was found to have precipitated from the water in solid form within 6 hours and so was largely unavailable to control algae and other aquatic pests. Total copper in 11 Sutter County soils ranged from 15 to 54 ppm and bioavailable copper from 4–10 ppm, amounting to 160 lbs of Cu per acre in 50 ppm fields..
4. The thin organic film (microlayer), which weighs only about 0.5 grams per square meter of water surface on a flooded rice field, concentrates applied hydrophobic pesticides to 10,000–100,000 times the levels found in the subsurface water. Concentrations of carbofuran (Furadan®) reached about

10,000 ppm (1% by weight) in the film, while thiobencarb (Bolero®) concentrations were even higher. These insecticides dissipated more slowly from films than from subsurface water, although photodegradation and transfer into water eventually reduced the pesticide concentrations. Microlayers were collected on silicone-treated glass plates, a revolutionary new technique.

5. A novel method has been developed for detection and measurement of fluorine-containing pesticides, such as diflubenzuron (Dimilin®), and their degradation products in soil and water. Called ^{19}F -NMR, it requires no extraction or sample cleanup and recognizes only the compounds of interest.
6. A full-scale field experiment on the distribution of a copper sulfate application to a flooded field proved useless, as the aerial applicator released virtually no pesticide. This suggests a need for some sort of future monitoring.

Donald G. Crosby

Principal Investigator

12/6/93

Date

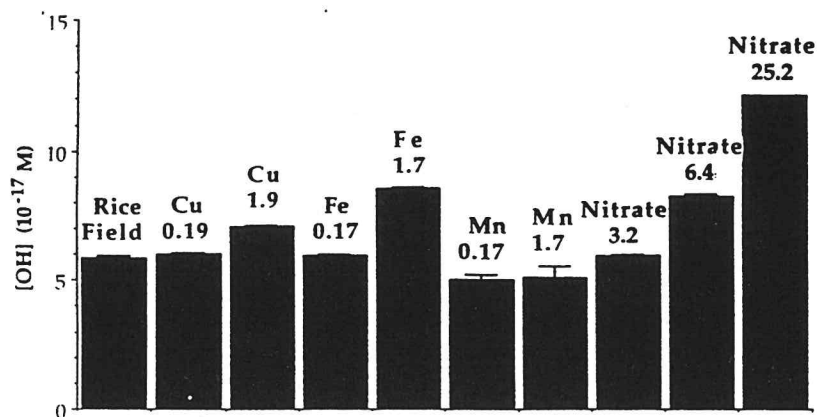


Fig. 1. Hydroxyl radical generation at various concentrations (mg/L) of metal ions and nitrate.

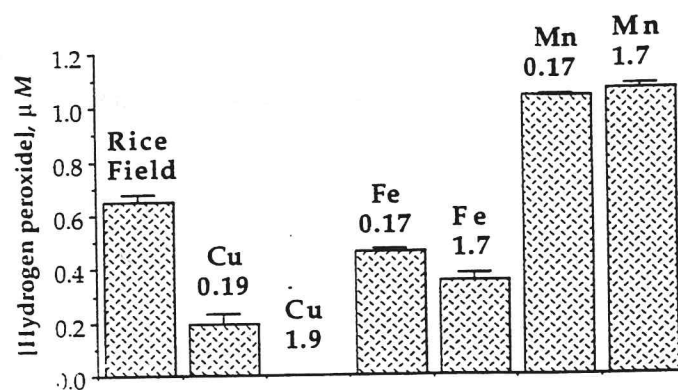


Fig. 2. Hydrogen peroxide generation in rice field water amended with metal ions at two concentrations (mg/L).

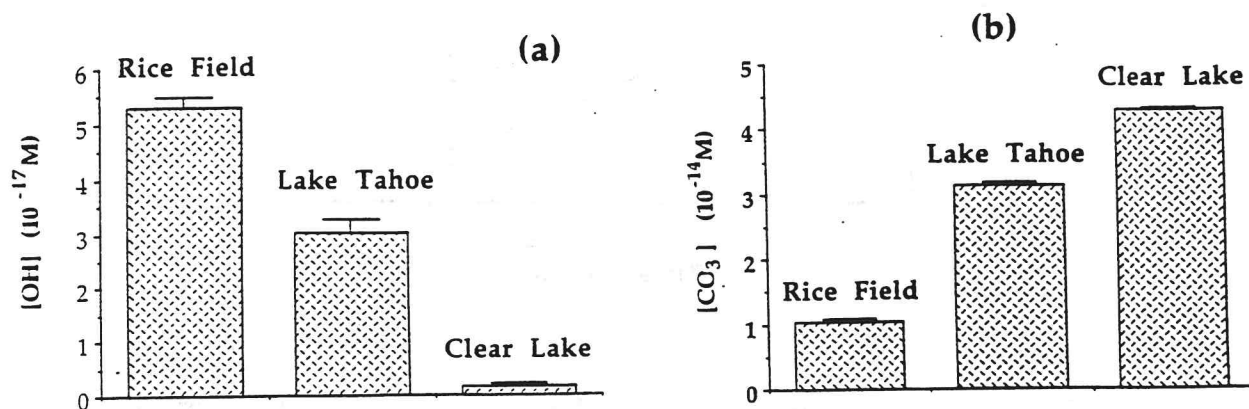


Fig. 3. Equilibrium concentrations of (a) hydroxyl and (b) carbonate radicals in three natural waters.

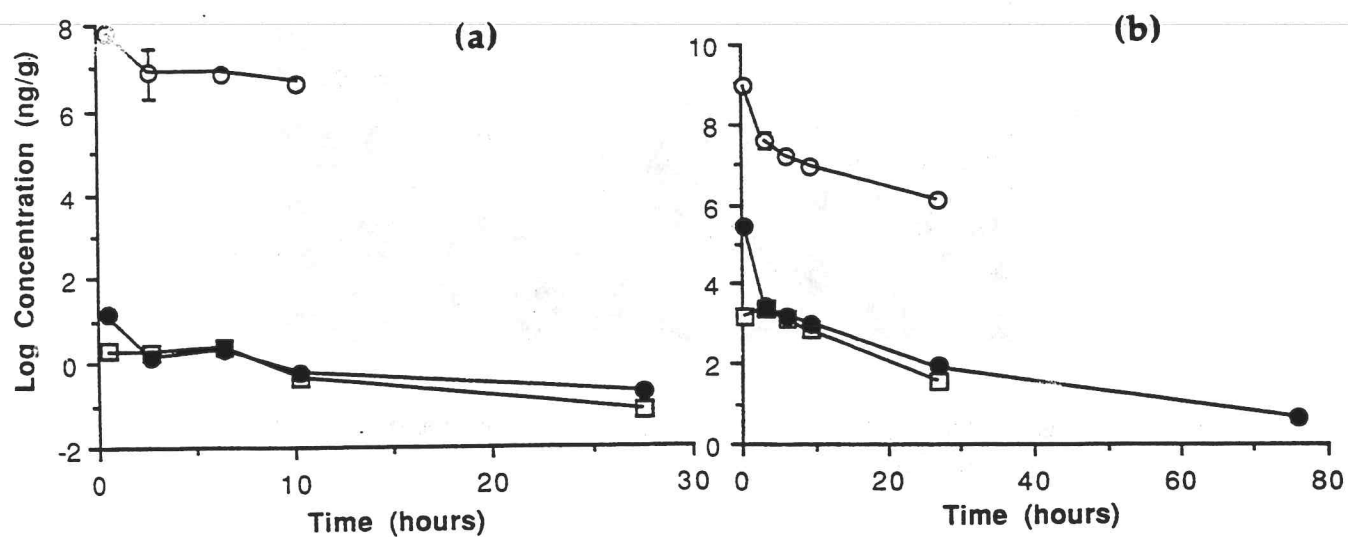


Fig. 4. Concentration of (a) carbofuran and (b) thiobencarb in surface microlayers. Silanized plates ○, unsilanized plates ●, subsurface water □.

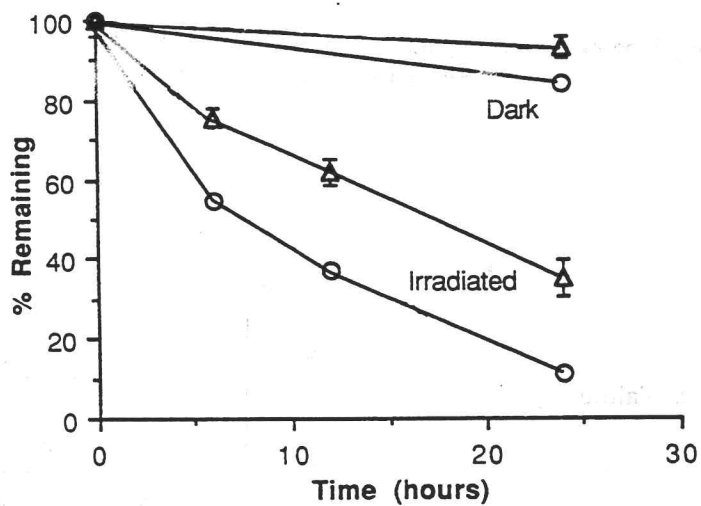


Fig. 5. Photodegradation of thiobencarb with (○) and without (Δ) microlayer.

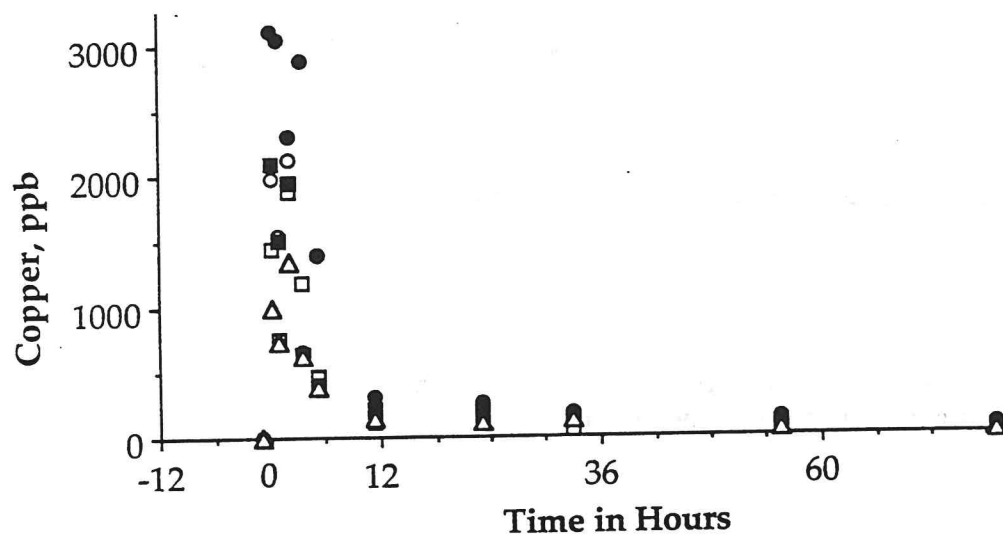


Fig. 6. Dissipation of copper from field water after aerial application at 15 lbs/acre (5 sites).
Outlet site \triangle

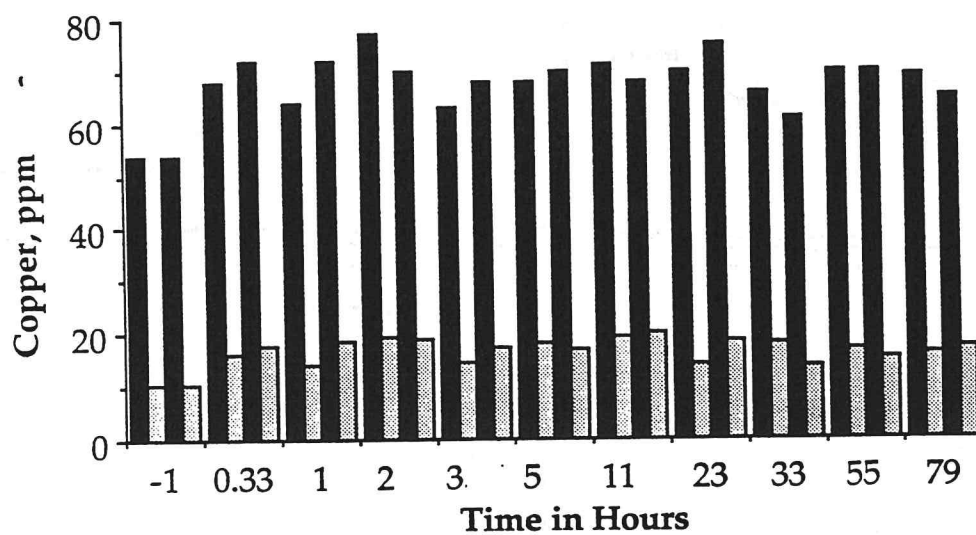


Fig. 7. Total copper \blacksquare and bioavailable copper \square at two sampling sites.

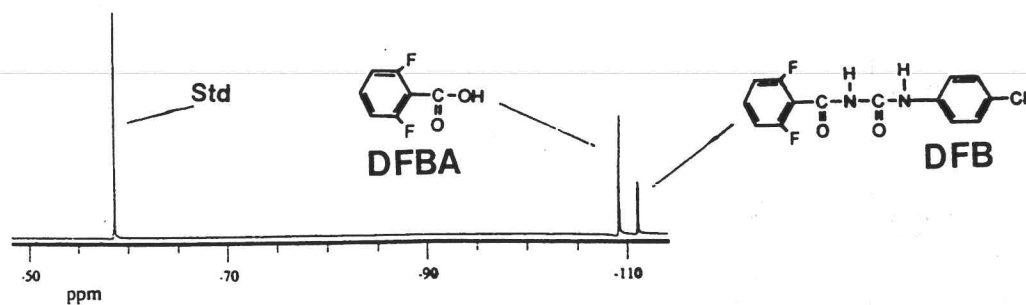


Fig. 8. ^{19}F -NMR signal of DFB and DFBA in a mixture, compared to that of an internal standard (4-trifluoromethoxyacetanilide).

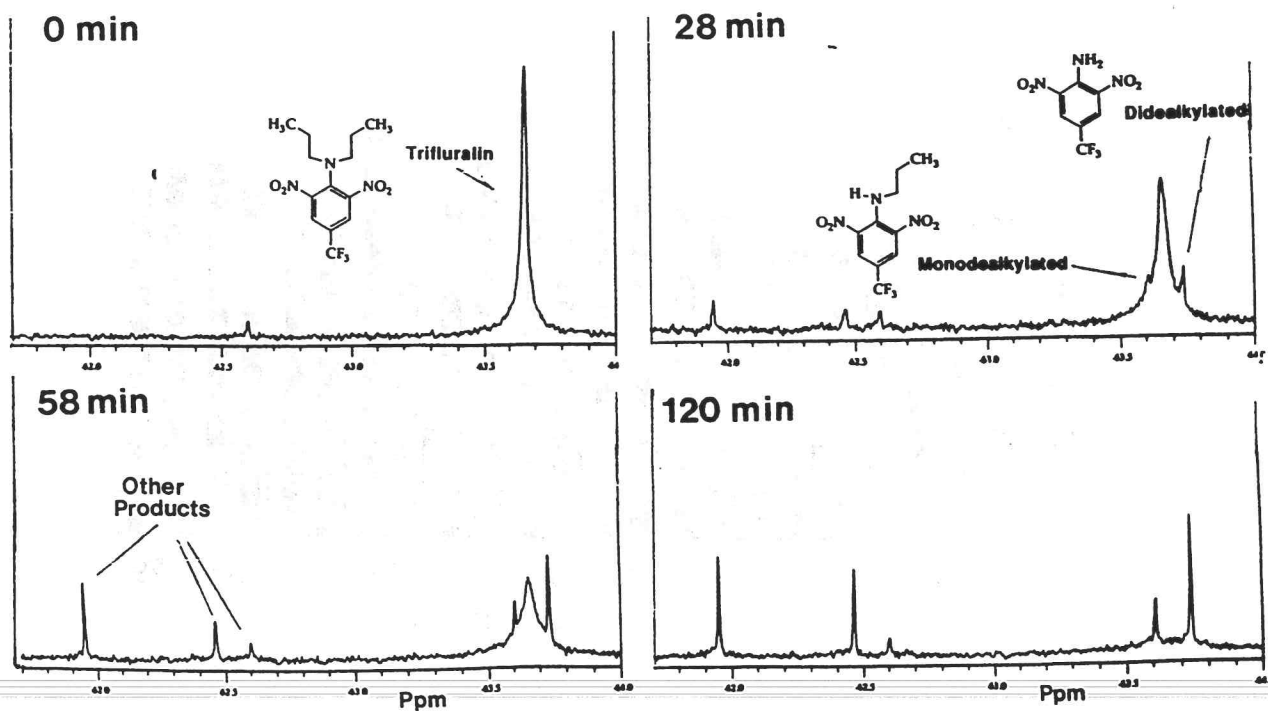


Fig. 9. ^{19}F -NMR spectra of photodegraded trifluralin in water.