

Novel Field Sampling Procedure for the Determination of Methiocarb Residues in Surface Waters from Rice Fields

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Methiocarb was extracted from surface water samples collected at experimental rice field sites in Louisiana and Texas. The sampling system consisted of a single-stage 90-mm Empore extraction disk unit equipped with a battery-powered vacuum pump. After extraction, the C-18 extraction disks were stored in an inert atmosphere at $-10\text{ }^{\circ}\text{C}$ and shipped overnight to the laboratory. The disks were extracted with methanol and the extracts analyzed by reversed-phase high-performance liquid chromatography with a methanol/water mobile phase. Methiocarb was detected by ultraviolet absorption at 223 nm and quantified with the use of calibration standards. Recoveries from control surface water samples fortified at 5.0, 10, 50, and 100 ng/mL methiocarb averaged $92 \pm 7\%$. A method limit of detection for methiocarb in rice field surface water was estimated to be 0.23 ng/mL at 223 nm.

Keywords: *Methiocarb; mesurol; high-performance liquid chromatography; solid phase extraction disks; water*

INTRODUCTION

Bird damage to seeded rice is a persistent problem in the southern United States that costs producers millions of dollars annually (1, 2). Currently, there is no commercially available effective bird repellent registered for rice seed. Wildlife biologists have been searching for an effective repellent to minimize the damage to rice for decades. Rice farmers have always had problems with bird depredation during planting season. Birds feed on the seed after surface water is removed during the seed soaking stage of germination. For a few days after water is removed from the fields, germinating rice seed is an attractive source of food to various bird species. After sprouts reach a couple of inches in height, the depredation subsides as the seed endosperm is no longer available to the birds as a source of food. Damage due to bird depredation is severe enough in some cases that nearly 100% of the rice seed is consumed and replanting is the only option.

Methiocarb [3,5-dimethyl-4-(methylthio)phenol methylcarbamate] has been known as an effective bird repellent for quite some time and is considered to be insoluble in water. Methiocarb causes gastrointestinal malaise for many avian species when ingested. Methiocarb is an *N*-methylcarbamate currently registered as a molluscicide. Methiocarb has been shown to be an effective bird repellent (3) when applied to rice seed in cage and pen trials (4). A seed treatment with methiocarb and a

Table 1. Ninety-Six Hour LC₅₀ Acute Oral Toxicity Data

species	mean (mg/L)	range (mg/L)
<i>Pteronarcys</i> (stoneflies)	0.005	0.004–0.006
rainbow trout	0.80	0.63–0.89
bluegill	0.21	0.12–0.36

commercially available adhesive were applied to rice seed to produce a 0.075% methiocarb-fortified rice seed, which was distributed in flooded fields aerially for efficacy trials. The fields remained flooded for three to six days for seed soaking and were then drained to allow germination to proceed. The water is typically drained back into rivers or nearby estuaries. Although methiocarb has a human health risk as it is a cholinesterase inhibitor and therefore can affect the nervous system, the risk as a seed treatment to humans would be extremely minimal. A more pressing concern would be the toxicity to aquatic organisms. As shown in Table 1 the acute oral toxicity for cold-water fish (rainbow trout) and warm-water fish (bluegill) is high. The acute oral toxicity for aquatic invertebrates is considered to be very high (5). Therefore, during the experimental phase of field efficacy trials a simple method to monitor the methiocarb residues in rice field surface waters was needed to supply data for potential registration inquiries and to ascertain the effectiveness of the adhesive used to limit the loss of methiocarb bound to the rice seed. Field trials in small test plots were set up for residue analysis in Louisiana and Texas.

Several methods for the determination of methiocarb in water are available. However, water samples of 500 mL in volume are expensive to ship and therefore a simple field extraction method was pursued that local operations personnel could use to collect samples.

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Improved sample preconcentration products called solid phase extraction (SPE) disks were introduced in 1989. This extraction technique combined with reversed-phase high-performance liquid chromatography (HPLC) and ultraviolet (UV) detection could yield a method to determine sub-parts per billion (ng/mL) levels of methiocarb. The 3M Co., Inc. (Minneapolis, MN), produces a solid phase extraction disk product called Empore Disks that have high sample throughput and efficiency. With flow rates up to 100 mL/min possible, the sample throughput is far superior to that of SPE columns or cartridges. The disks are 4.6–90 mm in diameter and can be obtained in a variety of sorbents. The sorbents are impregnated into a Teflon [PTFE, poly(tetrafluoroethylene)] substrate that has a high capacity and minimal channeling. The carbamate herbicide thiobencarb has been extracted from water and soil samples with the aid of SPE disks preceded by analysis with gas chromatography (6).

MATERIALS AND METHODS

Reagents. Methanol (Fisher Scientific, Denver, CO) was of liquid chromatography grade. Deionized water was purified using a Milli-Q water purification system (Millipore, Bedford, MA). Methiocarb (99%) was purchased from Chem Services, Inc. (West Chester, PA). The solvents for chromatographic analysis were degassed by bubbling helium through the solvents.

HPLC Parameters. The HPLC system consisted of a Hewlett-Packard 1090 liquid chromatograph (Palo Alto, CA) equipped with a diode array detector. Additionally, a Hewlett-Packard 1050 UV-visible variable-wavelength detector was used for additional sensitivity. The methiocarb was separated from matrix components with a 25 cm × 0.46-cm i.d. stainless steel analytical column packed with 5- μ m Keystone ODS/Hypersil at 35 °C. To prolong column lifetime, a 1.5 cm × 0.46 cm i.d. Keystone ODS/H (Bellefonte, PA) guard column was used. The samples were chromatographed with a methanol/water (70:30) mobile phase at 1.00 mL/min. Aliquots of 100 μ L were injected into the chromatographic system, and the chromatographic response was recorded at 223 and 268 nm.

For postcolumn derivatization a single-stage postcolumn addition of 50 mM potassium hydroxide, 0.10 mg/mL *o*-phthalaldehyde, and 0.72 mg/mL *N,N*-dimethyl-2-mercaptoethylamine hydrochloride (Thiofluor) was added at 0.200 mL/min. After addition of the derivatization reagent, the two streams were mixed in a beaded string reactor made with PTFE tubing (1 m × 0.50 mm i.d., Supelco, Bellefonte, PA) and heated to 120 °C in a Dionex (Sunnyvale, CA) 4-m reaction coil (500 μ L). It is reported that Thiofluor provides the best stability and is satisfactory for overnight chromatographic runs (7). The sensitivity of the method is reported to be equal to that of the two-stage method. This innovation simplifies reagent preparation and equipment maintenance while shortening start-up time without any loss in sensitivity (7, 8). A SpectroVision Inc. (Cambridge, MA) dual monochromator fluorescence detector placed serially in-line with the ultraviolet detector was used with an excitation wavelength of 338 nm and an emission wavelength of 424 nm. The methiocarb peak was identified by comparison with the retention time of a standard. A Hewlett-Packard computer work station with a printer was used to collect, process, store, and print the chromatographic data.

Sample Preparation. The extraction apparatus (Figure 1) consisted of a single-stage manifold, a 1000-mL reservoir, a disk support ring, a reservoir support, and C-18 disks purchased from the 3M Co. The vacuum pump (Barnant model 83041, 1/30 hp, 1650 rpm, Fischer Scientific, Inc., Denver, CO) was powered by a 12-V battery. Glassware such as beakers and graduated cylinders, glass fiber filters, and tubing were from VWR, Inc. (Denver, CO). The SPE disks were conditioned with two 10-mL portions of methanol followed by deionized

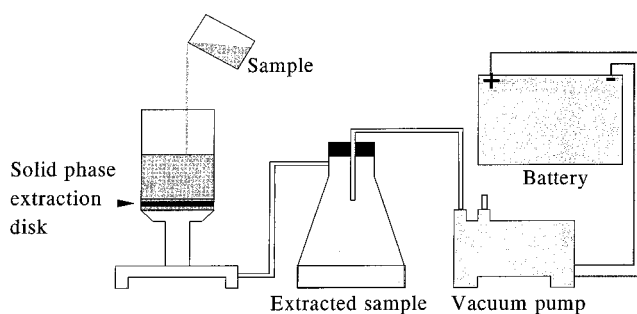


Figure 1. Field sampling extraction apparatus. The apparatus is very portable and compact, eliminating the need to ship water samples back to the laboratory.

Table 2. Recovery of Methiocarb from Surface Waters ($n = 6$ for All Levels)

fortification concn (ng/mL)	mean recovery (%)	SD (%)	CV (%)
5.0	89.7	8.2	9.1
10	91.2	6.9	7.6
50	90.8	7.5	8.3
100	94.0	7.2	7.6
grand mean	91.8	6.9	7.5

water. Each aliquot of methanol was allowed to equilibrate on the disk for 1 min. After the first aliquot, the disk was dried for 1 min by applying a vacuum. The second aliquot of methanol was followed by deionized water after the equilibration period. The disks were then rinsed with three 30-mL aliquots of deionized water. The water samples collected ranged in volume from 250 to 1000 mL. The sample extraction time was dependent on the percentage of particulate matter in the sample with 15 min as the maximum amount of time needed to extract a sample. For samples with visible particulate matter a 1- μ m glass fiber filter was placed in series with the extraction disk to reduce clogging of the disk. The water sample was acidified with 25 mL of a 0.001 M acetic acid solution to minimize hydrolysis of methiocarb. The sample was transferred to the extraction apparatus reservoir and the vacuum was initiated; the flow was maintained between 50 and 100 mL/min. The SPE disk was dried by drawing air for 5 min. The SPE disk was removed and carefully wrapped in aluminum foil, transferred to a resealable sample bag, and purged of air by allowing a small piece of dry ice (CO₂) to sublime and fill the sealed bag with gaseous carbon dioxide. The bag was opened to allow the gases to be expelled, two to three times. The sample extraction disks were placed in a cooler with ice, transferred to a freezer as soon as possible, and via overnight delivery shipped to the laboratory for analysis.

A 15-mL graduated centrifuge tube was placed below the SPE disk in the sample reservoir assembly to collect the extract. The SPE sample disk was placed in position, and 4 mL of methanol was added to the top of the disk. The saturated disk was allowed to equilibrate for 1 min, and then the eluant was removed by vacuum and the disk was air-dried for 1 min. The previous step was repeated two more times with 3-mL portions of methanol. The extract in the centrifuge tube was diluted to 10.0 mL with deionized water, vortex mixed, and transferred to a sample vial for HPLC analysis. The concentration of methiocarb was quantified by comparing the peak area response of a calibration standard to the peak area response of the analyte in the extract. Calibration standards ranged in concentration from 25 to 5000 ng/mL.

RESULTS AND DISCUSSION

Methiocarb in surface water was easily retained with C-18 SPE disks. For the range of 5–100 ppb of methiocarb, the mean recovery was 91.8 ± 6.9% (Table 2). The method limit of detection (MLOD) was 0.23 ng/mL at

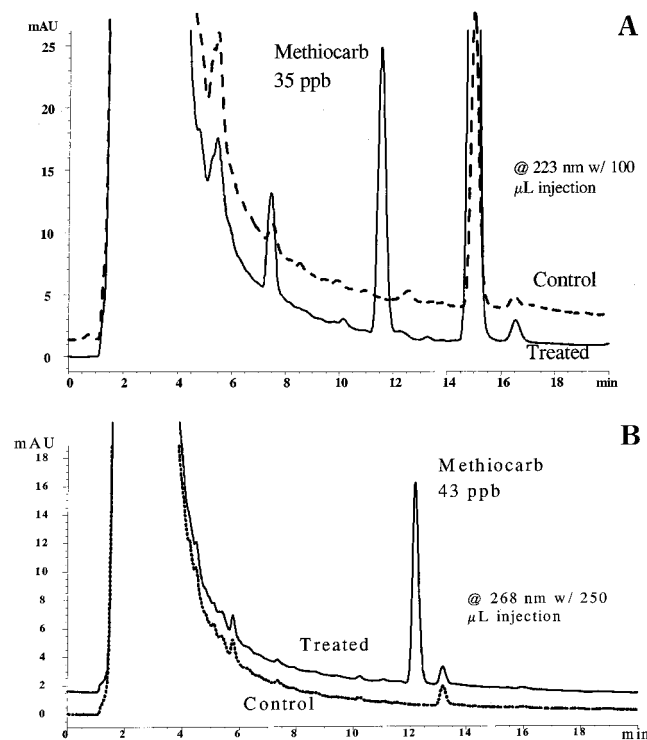


Figure 2. Chromatograms of surface water samples from a rice field planted with methiocarb-treated rice seed and untreated rice seed (control) extracted with the method and analyzed by HPLC utilizing UV detection at 223 (A) and 268 nm (B).

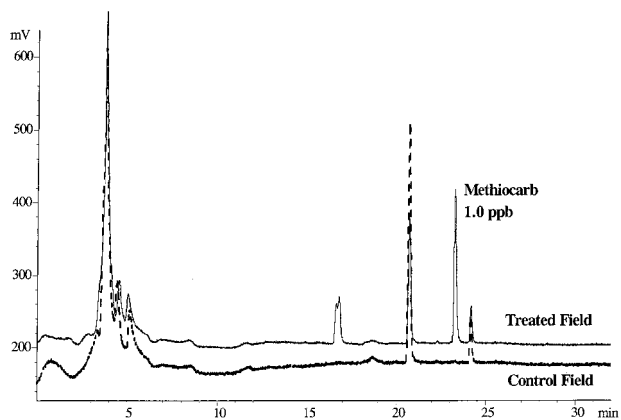


Figure 3. Chromatograms of surface water samples from a rice field planted with methiocarb-treated (1.0 ng/mL in the water sample) rice seed and untreated rice seed (control) extracted with the method and analyzed by HPLC utilizing postcolumn derivatization with fluorescence detection.

223 nm when the chromatographic response was 3 times the baseline noise (peak-to-peak) and methiocarb was extracted from 1000 mL of surface water. Chromatograms (Figure 2) recorded at 223 nm demonstrated a lower MLOD than those recorded at 268 nm, even when a larger injection volume (250 μ L) for detection at 268 nm was used. However, the chromatograms recorded at 268 nm were more selective for methiocarb as demonstrated by the simpler chromatogram from the control samples (Figure 2). With the use of preconcentration, samples were easily analyzed with HPLC and UV detection, instead of postcolumn derivatization with fluorescence detection. However, if a lower detection limit was required, this extraction method could be used with postcolumn derivatization and fluorescence detection, and the MLOD could be lowered to easily detect

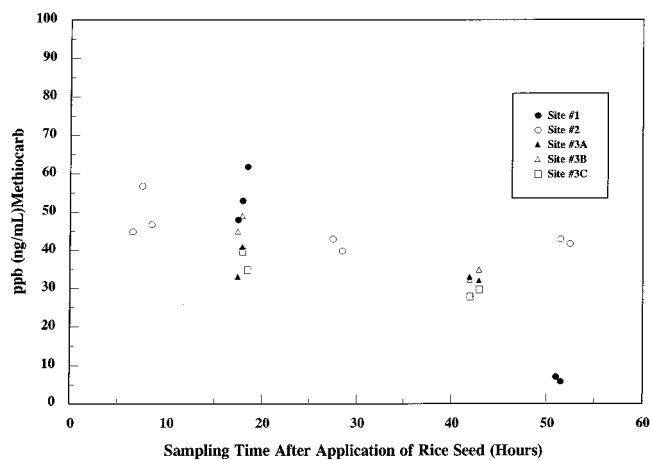


Figure 4. Methiocarb residues observed in surface waters from several locations over 6–52 h after seeding and the effect of pH.

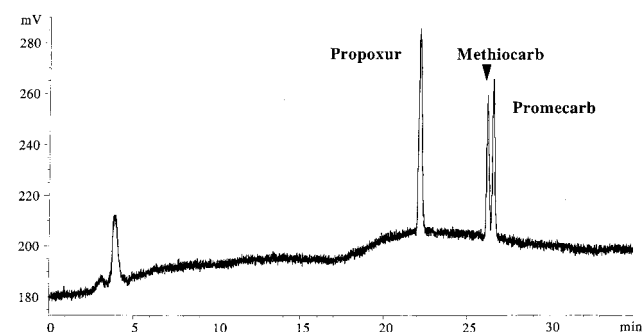


Figure 5. Chromatograms of a 0.10 ng/mL propoxur, methiocarb, and promecarb fortified control sample extracted with the method and analyzed by HPLC utilizing postcolumn derivatization with fluorescence detection.

sub-0.05 ng/mL levels for *N*-methylcarbamates as demonstrated in Figure 3.

The concentration of methiocarb residues from surface water samples at various field sites (five sites) appeared to be stable over a couple of days, in locations where the water had an approximate pH of 4–5 (Figure 4). However, at one field site the pH was measured between 6 and 7, and the methiocarb residues decreased noticeably with time at this location. This is not unexpected as methiocarb is readily hydrolyzed with increasing pH. The half-life of methiocarb was 321 days at pH 5, 24 days at pH 7, and 0.2 days at pH 9 (personal communication, unpublished data, Gowan, Inc., Yuma, AZ).

The method proved to be very simple and was also adaptable to other carbamates (Figure 5) such as propoxur, methiocarb, and promecarb, which were determined simultaneously in water. These analytes were detected by postcolumn derivatization with fluorescence detection just to demonstrate the potential of the extraction technique.

In conclusion, the analytical procedure from extraction of surface water samples in the field to analysis of the disks in the laboratory was very simple with high recoveries and reproducibility. The field extraction procedure could be completed by local operators with some basic training. The SPE disks were very durable to heat and cold and were extremely light. The samples could be analyzed the next day in the laboratory with overnight delivery. The method was inexpensive and very rapid. Multiple samples could be collected simultaneously in the field as one individual could easily

monitor and operate three to four sampling devices at a time.

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