Determination of the Aquatic Herbicide Fluridone in Water and Hydrosoil: Effect of Application Method on Dissipation

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The rate of fluridone dissipation from the water and hydrosoil of two ponds following application of the aquatic herbicide by two different methods was found to be similar. The half-life of fluridone in water was 21 and 26 days following application to the surface of the water and along the bottom of the pond, respectively. The residue pattern of fluridone on the hydrosoil was also found to be similar in both ponds, with no detectable residue remaining 56 days after treatment. Residue determinations were accomplished by reverse-phase high-pressure LC with UV detection at 254 nm. Water samples were filtered and injected directly into the high-pressure LC or were extracted and concentrated prior to high-pressure LC analysis. Hydrosoil extracts were purified by XAD-2 and alumina column chromatography prior to high-pressure LC analysis.

Fluridone (1-methyl-3-phenyl-5-[3-(trifluoromethyl)-phenyl]-4(1*H*)-pyridinone) is an experimental herbicide which has provided control of several vascular aquatic weeds when applied either to the water surface or along the bottom of weed-infested ponds (McCowen et al., 1979; Grant et al., 1979; Rivera and West, 1979; Parka et al., 1978). The presence of a pesticide in water can be a route for human exposure directly via consumption of water and fish or indirectly via consumption of irrigated crops or animal food products from livestock and poultry exposed to the water. Consequently, the rate of pesticide dissipation is important for determining if time restrictions are needed on the use of the water following treatment. The residue pattern of fluridone in aquatic environments has been determined from experiments conducted in several geographic regions of the United States (West et al., 1979) and in Canada (Muir et al., 1980). However, the effect of different application techniques on fluridone dissipation has not been studied under closely controlled field conditions.

The placement of the herbicide at different depths in the water column could potentially affect rates of fluridone photolysis, adsorption onto hydrosoil, and plant uptake. These are factors which are known to contribute to the dissipation of fluridone from treated water (West et al., 1979). In the study reported here, efforts were made to eliminate or minimize variables other than application technique which could affect the dissipation of fluridone. These variables included the herbicide formulation, the rate and date of treatment, geographic location, weather patterns, depth, size, and shape of the ponds, and residue sampling techniques. These variables were standardized by treating two similar, adjacent ponds with an aqueous suspension of fluridone at the same rate and then collecting residue samples from both ponds in an identical manner.

The residue data were generated by newly developed methods utilizing reverse-phase high-pressure LC with UV detection at 254 nm. These methods represent improvement over previous methods (West, 1978) by eliminating the need to brominate fluridone for detection by electron-capture gas chromatography. The new procedures have resulted in decreased sample analysis time and improved analytical precision.

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Table I. Cations and Total Organic Carbon in Pond Water

	concn, ppm, in water	
	surface-application pond	bottom-application pond
Mg^{2+}	13	23
Ca^{2+}	30	53
NH_4^+	0.10	0.19
Na^+	11	18
Fe^{2+}	0.036	0.006
total carbon	11	12

EXPERIMENTAL SECTION

Pond Description. Two adjacent man-made ponds in central Indiana were utilized for determining the effect of application technique on fluridone dissipation. Both ponds were rectangular in shape with dimensions of 38 × 98 m, equaling 0.37 ha in size. The bottom of both ponds sloped gradually from a shallow end (0.75-m water depth) to a deep end (1.5-m water depth), with an overall average water depth of 0.9 m. On the day prior to treatmtent, the water temperature of both ponds at 6 in. below the water surface was 24.1 °C, and the air temperature was 22 °C. The pH of the pond which received a surface application of fluridone was 8.5, while the pH of the other pond was 8.0. The relative amounts of cations and total organic carbon in the water of the two ponds are summarized in Table I. The water level in the ponds was regulated by a constant level drain and water pumped in from a holding pond.

Herbicide Application. Fluridone formulated as a 4 lb/gal aqueous suspension (4AS) was applied at 0.84 kg of active ingredient/ha of surface water on June 13, 1979. Application of the 4AS was made from a boat with a sprayer consisting of a five-roller pump driven by a 3.5-hp engine. In one pond, the 4AS was applied to the surface of the water with a hand-held spray gun. In the second pond, the 4AS was layered along the bottom of the pond by using two trailing, weighted hoses with three 0.16-cm orifices. Both applications were made by diluting the 4AS formulation with water to a volume of 476 L, which was then applied to the pond at a rate of 7.6 L/min at 2 atm of pressure.

Residue Sampling Procedures. Residue samples were collected from both ponds at regular intervals (see Table II) to determine the dissipation rate of fluridone from the water and hydrosoil. Water subsamples (~320 mL) were collected from the midpoint of the water column with a Kimmerer sampling apparatus (Lind, 1979) at nine loca-

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Table II. Influence of Application Method on Fluridone Dissipation from Ponds Treated with 0.84 kg of Fluridone/ha

	residue from surface application			residue from bottom application		
	ppm in water		kg/ha in	ppm in water		kg/ha in
DAT[a]	extraction	direct injection	hydrosoil	extraction	direct injection	hydrosoil
1	0.100		0.02	0.081		NDR[b]
3	0.114			0.093		
7	0.058	0.067		0.050	0.057	
14	0.061	0.058	0.08	0.043	0.045	0.06
20	0.044	0.042		0.038	0.038	
28	0.037	0.037	0.04	0.040	0.033	0.03
56	0.010	0.011	NDR	0.015	0.013	NDR
110	0.004	0.006	NDR	0.013	0.006	NDR

[a] Days after treatment. [b] No detectable residue at a test sensitivity of ~0.01 kg/ha.

tions in each pond. The subsamples were placed in a cooler with ice and delivered to the analytical labroatory, where they were composited and stored at 4 °C until analyzed. Nine hydrosoil subsamples were collected to a depth of ~15 cm with a soil sampler containing a removable 2.5 cm i.d. plastic tube (Giddings Machine Co., Ft. Collen, CO). The plastic tubes containing hydrosoil were capped and placed in a cooler with ice for transport to the laboratory, where the subsamples were combined and excess water was removed by vacuum filtration. After the soil was air-dried for 2–3 days, it was ground, blended, weighed, and stored at 4 °C until analyzed.

Storage Stability Study. A study was conducted to determine the stability of fluridone in pond water during storage at 4 °C for several weeks prior to analysis. One liter of control pond water was fortified in duplicate with 1.0 ppm of fluridone and was preserved with 0.5 mL of concentrated sulfuric acid to prevent microbial degradation. Control pond water fortified with 1.0 ppm of fluridone but lacking the acid preservative was also prepared in duplicate. The samples were stored in plastic containers in the dark at 4 °C and were periodically assayed.

Apparatus, Chemicals, and Reagents. Water and methanol for the high-pressure LC mobile phase and for preparing analytical standards were high-pressure LC grade (J. T. Baker or Waters Associates); hexane was omnisolve grade, glass distilled (MCB); all other solvents were reagent grade. Dichloromethane was redistilled. Anhydrous sodium sulfate was washed with reagent-grade methanol and dried at 50 °C for 16 h. Alumina (Alcoa F-20) was deactivated with 4.0% water (v/w) and tumbled for 1 h in a closed container. Amberlite XAD-2 synthetic ion-exchange resin (Rohm and Haas) was successively washed with deionized water, methanol, and acetone until all traces of oily residue were removed from the resin and the supernatant liquid above the resin was no longer cloudy. The resin was then added as a slurry in deionized water to a height of 20 cm in a chromatography column (250 × 14 mm i.d.) containing a glass wool plug and equipped with a stopcock and a 250-mL reservoir. The column was topped with a glass wool plug, and the resin was washed with 200 mL of acetone, followed by 200 mL of methanol and 200 mL of 0.01 N sodium hydroxide, which was drained to 5 cm above the resin. The column was capped until used.

Measurement of residues was accomplished by high-pressure LC using a Waters Model 6000A solvent delivery system, a Waters Model 440 absorbance detector operated at a fixed wavelength (254 nm), a Waters Model 710A Intelligent sample processor, and a Houston Instruments Omni Scribe strip chart recorder. The column was a μBondapak C_{18} (3.9 mm × 30 cm) with a CO–PELL ODS guard column (Whatman, Inc.). The mobile phase consisted of methanol–water (60:40) at a flow rate of 1.1 mL/min. The chart speed was 0.25 cm/min. Injection volumes were 200 or 1000 μL, and the attenuation was 0.01 or 0.05 AUFS.

Residue Analysis. (*A*) *Water.* The concentration of fluridone in pond water was determined by two different techniques. The first technique (referred to as direct-injection high-pressure LC) involved filtering ~15 mL of a water sample through folded filter paper (Schleicher & Schuell, 12.5 cm, No. 588) into a screw-cap bottle. An aliquot of the filtered sample was transferred to an high-pressure LC sample vial, which was then fitted with a self-sealing septum cap. The filtered solution was injected (1000 μL) directly into the high-pressure LC at a sensitivity of 0.01 AUFS. A direct standard consisting of 0.05 μg/mL fluridone in high-pressure LC grade water was also injected (1000 μL) for quantitative measurements.

The second technique for water analysis (referred to as extraction high-pressure LC) involved the extraction of 100 mL of pond water with three 20-mL aliquots of dichloromethane. The dichloromethane extracts were dried and combined by draining through a funnel containing sodium sulfate into a 125-mL evaporating flask. The dichloromethane was evaporated to dryness with a rotary vacuum evaporator and a 40 °C water bath. The residue was dissolved in 2.0 mL of high-pressure LC grade methanol–water (60:40) and transferred to a high-pressure LC sample vial which was then fitted with a self-sealing septum cap. The concentrated water extract was injected (200 μL) into the high-pressure LC at a sensitivity of 0.05 AUFS. A direct standard consisting of fluridone at 1.0 μg/mL in high-pressure LC grade methanol–water (60:40) was also injected (200 μL) for quantitative measurements.

(*B*) *Hydrosoil.* A representative 25-g hydrosoil sample (prepared as described previously) was weighed into a 0.5-L jar, and 100 mL of 2 N sodium hydroxide–methanol (50:50) was added. The fluid level was accurately marked on the jar with waterproof ink, and the jar was covered with a watch glass and placed in a 90–95 °C water bath. After the sample was boiled for 30 min, 2 N sodium hydroxide–methanol (50:50) was added to reestablish the original fluid level. After the mixture was boiled for an additional 30 min, the jar was removed from the water bath and the contents were cooled to room temperature. Methanol was added to reestablish the original fluid level, and the jar was swirled to thoroughly mix the contents. A 20-mL aliquot of the hydrosoil extract was collected by pouring the supernatant liquid through a funnel containing folded filter paper (Schleicher & Schuell, No. 560) into a graduated cylinder. The 20-mL aliquot was transferred to a chromatography column containing XAD-2 resin prepared as described previously. The eluant was drained to the top of the resin, and the eluate was discarded. The graduated cylinder was rinsed twice with 5-mL aliquots of 0.01 N sodium hydroxide–methanol (90:10). Each rinse was added

Table III. Recovery and Precision of Residue Methods for Trace Levels of Fluridone in Pond Water and Hydrosoil

sample type	HPLC method	ppm of fluridone added	no. of replicates	% recovery		
				range	av	coeff of var.
pond water	direct injection	0.05	8	90-100	95	3.5
		0.100	6	96-100	97	2.1
		1.000	8	99-102	100	1.8
pond water	extraction	0.001	9	105-149	125	14.7
		0.010	9	84-113	101	8.7
		0.100	6	86-100	92	6.0
hydrosoil	extraction	0.010	6	80-100	97	12.3
		0.100	7	71-89	79	9.2

separately to the column and drained to the top of the resin, and the eluate was discarded. The column was washed with an additional 50 mL of 0.01 N sodium hydroxide–methanol (90:10), and the eluate was discarded. The column was washed with 80 mL of 0.01 N sodium hydroxide-methanol (50:50) and the eluate was discarded.

Fluridone was eluted from the column with 80 mL of methanol, and the eluate was collected in a clean beaker. (The XAD-2 resin was regenerated for future use by washing with 100 mL of acetone, followed by 100 mL of methanol and 100 mL of 0.01 N sodium hydroxide, which was drained to 5 cm above the resin.) The methanol eluate containing the fluridone residue was transferred to a 250-mL separatory funnel containing 100 mL of 5% sodium chloride solution. The aqueous phase was extracted 3 times with 40-mL aliquots of dichloromethane. The dichloromethane extracts were dried and combined by passing through a funnel containing sodium sulfate into a 250–mL evaporating flask. The combined extract was evaporated to dryness by using a rotary vacuum evaporator and a 40 °C water bath. The residue was dissolved in 5 mL of hexane–dichloromethane (70:30). The hydrosoil extract was added to a chromatography column prepared by wet packing 10 mL of 4% water-deactivated alumina in hexane-dichloromethane (70:30) and topping the column with a 1-cm layer of anhydrous sodium sulfate. (Prior to initial use, the alumina was standardized to determine the elution pattern of fluridone.) The extract was drained to the top of the sodium sulfate, and the eluate was discarded. The flask was rinsed twice with 5-mL aliquots of hexane–dichloromethane (70:30), each rinse was added separately to the column, and the eluate was discarded. The column was washed with an additional 25 mL of hexane–dichloromethane (70:30), followed by 20 mL of dichloromethane, and the eluates were discarded. Fluridone was eluted from the column with an additional 50 mL of dichloromethane, and the eluate was collected in a 125-mL evaporating flask. The dichloromethane was evaporated to dryness by using a rotary vacuum evaporator and a 40 °C water bath. The residue was dissolved in 2.0 mL of high-pressure LC grade methanol–water (60:40) and transferred to an high-pressure LC sample vial, which was then fitted with a self-sealing septum cap. The concentrated hydrosoil extract was injected (200 μL) into the high-pressure LC at a sensitivity of 0.01 AUFS. A direct standard consisting of fluridone at 0.25 μg/mL in high-pressure LC grade methanol–water (60:40) was also injected (200 μL) for quantitative measurements.

Calculations. Analytical results were expressed in terms of ppm (μg/mL) of fluridone in water and kg/ha fluridone in hydrosoil in order to relate residue levels in hydrosoil to the application rates (West et al., 1979).

RESULTS AND DISCUSSION

The dissipation of fluridone from the water and hydrosoil of both ponds is summarized in Table II. The data indicate that fluridone dissipated at a similar rate with both application techniques. Half-lives determined from a least-squares line obtained by plotting the concentration of fluridone vs. the number of days after treatment on a semilogarithmic scale were 21 and 26 days for the surface and bottom applications, respectively. Only 4% of the initial fluridone concentration remained in the water of either pond 110 days after treatment.

The residue pattern of fluridone in the hydrosoil was also similar for both types of application. In both ponds, maximum fluridone residues in the hydrosoil were observed 14 days after treatment and were equivalent to 7 and 9% of the total amount of fluridone applied to the ponds. Fluridone was not detected in the hydrosoil of either pond 56 days after treatment.

Thus, the method of applying fluridone to the pond did not appear to affect the dissipation of the herbicide from the aquatic environment. Variations in the rates of photolysis, hydrosoil adsorption, and plant uptake which could occur if fluridone were to remain concentrated at the top or bottom of the water column are apparently minimized by a rapid dispersal of the herbicide throughout the entire water column following application of the 4AS formulation (West et al., 1979; Rivera and West, 1979).

As indicated in Table II, both analytical methods for determining the concentration of fluridone in water gave similar results. The difference in assay results obtained from the direct-injection and extraction high-pressure LC techniques averaged 0.003 ppm. The direct-injection high-pressure LC technique was more precise than the extraction technique, as shown in Table III for untreated control water fortified with trace levels of fluridone. Both methods result in greater precision and are less time consuming than the derivatization of fluridone for detection by gas chromatography with electron-capture detection (GC-ECD) (West, 1978).

The extraction high-pressure LC and GC-ECD techniques are both capable of detecting trace levels of fluridone as low as 1 ppb. The direct injection high-pressure LC technique has a detection limit of ~5 ppb, and attempts to improve the sensitivity by injecting sample volumes greater than 1000 μL were unsuccessful due to peak broadening. Unacceptable peak broadening was also observed when 1000 μL of the fluridone standard dissolved in the mobile phase was injected. Consequently, fluridone standards were diluted to the desired concentrations with high-pressure LC grade water. Chromatograms demonstrating the determination of fluridone concentration in water are presented in Figures 1 and 2.

Recoveries of fluridone from untreated hydrosoil fortified at the 0.010- and 0.100-ppm level averaged 97 and 79% with coefficients of variation of 12.3 and 9.2%, respectively. The residue results for fluridone in a treated hydrosoil sample assayed in triplicate on 2 consecutive days ranged from 20 to 27 ppb with an average of 23 ± 2 ppb.

As was the case with water, the high-pressure LC technique for hydrosoil is also more precise and less time consuming than the derivatization of fluridone for GC-

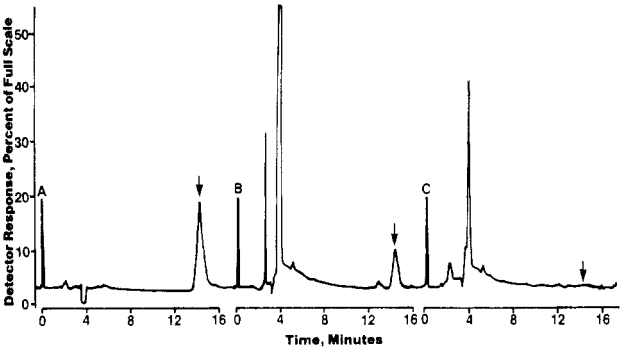

Figure 1. High-pressure liquid chromatograms demonstrating the recovery of fluridone from pond water by using the extraction high-pressure LC method: (A) fluridone standard, 200 ng; (B) untreated pond water fortified with 0.01 ppm of fluridone, equivalent to 92% recovery; (C) untreated pond water. (Arrows indicate retention time of fluridone.)

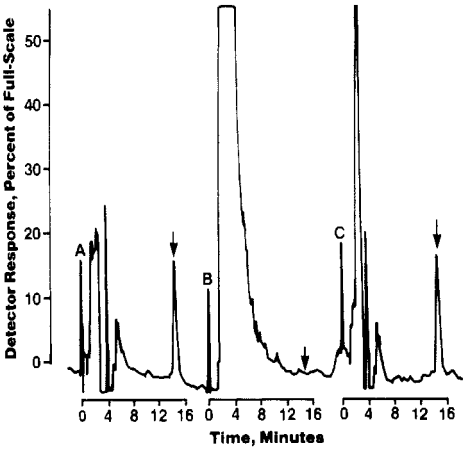

Figure 2. High-pressure liquid chromatograms demonstrating the recovery of fluridone from pond water by using the direct-injection high-pressure LC method: (A) fluridone standard, 50 ng; (B) untreated pond water; (C) untreated pond water fortified with 0.05 ppm of fluridone, equivalent to 100% recovery. (Arrows indicate retention time of fluridone.)

Table IV. Stability of Fluridone in Pond Water Stored in the Dark at 4 °C

storage time, days	fluridone concn, ppm			
	acid preserved		nonpreserved	
	no. 1	no. 2	no. 3	no. 4
0	0.99	0.91	0.99	0.85
6	1.03	1.01	1.03	1.05
10	1.10	1.13	1.08	1.08
29	1.13	1.12	1.06	1.12
51	1.02	1.01	1.00	0.98
113	0.70	0.70	0.72	0.64
174	0.79	0.81	0.79	0.76

ECD (West, 1978). The high-pressure LC technique is capable of detecting fluridone in hydrosoil at levels as low as 0.01 ppm, which matches the sensitivity of the derivatization method. Chromatograms demonstrating the recovery of fluridone from untreated hydrosoil fortified with 0.01 and 0.10 ppm of the herbicide are presented in Figure 3.

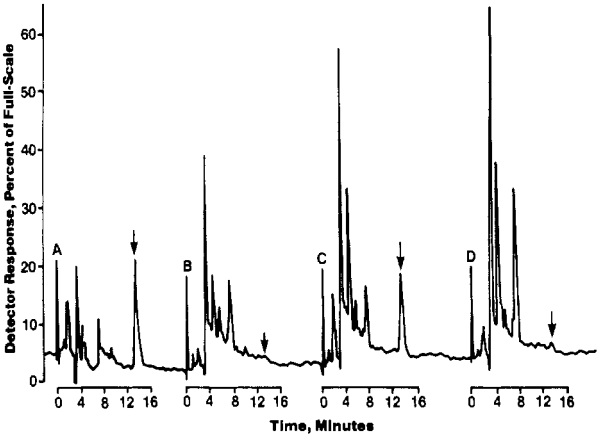

Figure 3. High-pressure liquid chromatograms demonstrating the recovery of fluridone from pond hydrosoil: (A) fluridone standard, 50 ng; (B) untreated hydrosoil extract; (C) untreated hydrosoil fortified with 0.10 ppm of fluridone, equivalent to 75% recovery; (D) untreated hydrosoil fortified with 0.01 ppm of fluridone, equivalent to 99% recovery. (Arrows indicate retention time of fluridone.)

The results of a stability study for fluridone in pond water samples stored at 4 °C are summarized in Table IV. Over a period of 51 days, no loss of fluridone was observed. However, after 113 days, the fluridone concentration decreased to an average of 74% of the initial values in both the acid-preserved and unpreserved samples. These data suggest that water samples containing fluridone should not be stored for longer than 2 months prior to analysis without accompanying storage stability samples. Since all water samples in the study were assayed within 7 days after collection, the resulting data accurately reflect the dissipation of fluridone from the pond water.

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