abolic alteration pathway but do not accumulate the desmethyl derivative.

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Fate of Fluridone in Sediment and Water in Laboratory and Field Experiments

Derek C. G. Muir* and Norbert P. Grift

The fate of the aquatic herbicide fluridone [1-methyl-3-phenyl-5-[(3-trifluoromethyl)phenyl]-4(1*H*)-pyridinone] was studied in sediment–water systems in culture flasks, in pond water exposed to sunlight, and in small ponds by using either a carbonyl-^{14}C- or *N*-methyl-^{14}C-labeled compound. The half-life of fluridone in sediment was 12 months under laboratory conditions (25 °C) and about 17 weeks under field conditions. In the laboratory study, the major degradation product of fluridone in sediment was fluridone–acid [1,4-dihydro-1-methyl-4-oxo-5-[3-(trifluoromethyl)phenyl]-3-pyridinecarboxylic acid; II]. II accounted for 48–54% of the radioactivity that was extracted from sediments incubated for a 26-month period. Two phenolic compounds, 4-hydroxyfluridone [1-methyl-3-(4-hydroxyphenyl)-5-[3-(trifluoromethyl)phenyl]-4(1*H*)-pyridinone; III] and the 2-hydroxy derivative (V), were identified as minor breakdown products of fluridone in sediment (0.5–2.5% of radioactivity). Desphenylfluridone [1-methyl-3-[3-(trifluoromethyl)phenyl]-4(1*H*)-pyridinone; I], II, III, and V were identified in aqueous solutions of fluridone held in Pyrex flasks in sunlight. The half-life of fluridone in ponds treated at 100 μg/L was 2–3.5 days, and III and V were identified at low levels (0.05–0.5μg/L) in water sample extracts. No major degradation products of fluridone were identified under field conditions due to the apparent extensive photodegradation of the compound.

Fluridone [1-methyl-3-phenyl-5-[3-(trifluoromethyl)-phenyl]-4(1*H*)-pyridinone] is an experimental aquatic herbicide for the control of vascular aquatic plants (Arnold, 1979; Dechoretz and Frank, 1978). It has also been tested for weed control in cotton (Webster et al., 1977). Studies on the disappearance of fluridone in aquatic systems have indicated that it has an average half-life of about 5 days in pond and lake waters (West et al., 1979; Muir et al., 1980). Dissipation of the herbicide in sediments and terrestrial soils under field conditions has been reported to be much slower than in water. Half-lives of fluridone in clay and fine sandy loam soils ranged from about 50–100 days (Banks et al., 1979). In sediments half-lives ranging from about 3 months to greater than 12 months have been observed (West et al., 1979; Muir et al., 1980).

No major degradation products of fluridone were found in water or sediment sample extracts from ponds treated at recommended rates of application (West et al., 1979; Muir et al., 1980) despite the overall rapid disappearance of the herbicide. It seemed likely that the degradation products were either extremely labile in water or that they could not be determined by the analytical techniques used in the field studies. Laboratory studies have shown that fluridone degrades rapidly in deionized water (half-life of 23 h) under artificial sunlight (West et al., 1979). Thus, photolysis may be a major pathway for disappearance of fluridone in water.

The purpose of the present work was to identify the major degradation products of fluridone in sediment and water by using laboratory studies and to compare the results with those of field studies on the disappearance of fluridone in small artificial ponds.

MATERIALS AND METHODS

Analytical Standards. Fluridone, 4-hydroxyfluridone [1-methyl-3-(4-hydroxyphenyl)-5-[3-(trifluoromethyl)-phenyl]-4(1*H*)-pyridinone], fluridone–acid [1,4-dihydro-1-methyl-4-oxo-5-[3-(trifluoromethyl)phenyl]-3-

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pyridinonecarboxylic acid], desphenylfluridone [1-methyl-3-[trifluoromethyl)phenyl]-4(1*H*)-pyridinone], and [*carbonyl*-^{14}C]fluridone (sp act. 17.89 μCi/mg) were gifts of Lilly Research Laboratories, Greenfield, IN. [*N-methyl*-^{14}C]Fluridone was synthesized as follows: *N*-desmethylfluridone [3-phenyl-5-[3-(trifluoromethyl)-phenyl]-4(1*H*)-pyridinone] (10 mg in 1.0 mL of hexane) was mixed with [^{14}C]methyl iodide (sp act. 116.5 μCi/mg) (2.2 mg), 20 mg of sodium hydride, and 1.0 mL of dimethyl sulfoxide in a test tube. The tube was sealed and heated at 60 °C for 1 h. The mixture was then cooled, water (1.0 mL) was added to remove sodium hydride, and the product was extracted with hexane (2 mL). The hexane extract was purified by chromatography on a small column, 7 cm × 6 mm i.d. of alumina (Basic Woelm; activated by heating at 110 °C) to remove unreacted *N*-desmethylfluridone and by thin-layer chromatography (TLC) (silica gel; 0.25 mm) using toluene–acetonitrile–acetic acid (65:35:1) as the solvent system. Purity of the product, as determined by gas chromatography and TLC-autoradiography, was >99%. Specific activity of [*N-methyl*-^{14}C]fluridone was 11.0 μCi/mg.

Fluridone, 4-hydroxyfluridone, fluridone–acid, and desphenylfluridone were prepared in methanol (10 mg/50 mL) and were diluted with methanol or ethyl acetate for the preparation of working standards. For degradation studies [^{14}C]fluridone was diluted with nonradiolabeled fluridone in acetone before addition to incubation flasks or pond water.

Apparatus. Gas chromatography (GLC) was carried out on a Tracor Model 560 equipped with a nitrogen–phosphorus detector. Columns of 5% OV-1 or 3% OV-17, both on Chromosorb W-HP (80–100 mesh) (0.6 m × 4 mm i.d. glass), were operated at 250 °C. Injector and detector oven temperatures were 250 and 260 °C, respectively.

High-pressure liquid chromatography (HPLC) was carried out by using a Waters Model 4000A pump and Model 440 UV detector at 254 or 313 nm. A reverse-phase column (Altex Ultrasphere ODS) (25 cm × 4.6 mm i.d.) and a guard column (4 cm × 4.6 mm i.d.; Bio-Rad Laboratories, Richmond, CA) were used with a solvent system consisting of water–methanol–formic acid (40:60:0.2). Solvent flow rate was 1.5 mL/min.

Sample extracts containing [^{14}C]fluridone were dissolved in 12.5 mL of PCS (Amersham Corp., Oakville, Ontario, Canada)–xylene (Fisher; scintanalyzed grade) (2:1) and counted on either a Beckman 7500 or a Packard 3030 scintillation counter.

Combustion of sediment samples was carried out on a Packard 306 oxidizer. $^{14}CO_2$ was collected in CO_2-M-Met (Amersham) and diluted with PCS in the instrument. The samples were counted by scintillation counting as described.

TLC was performed by using silica gel plates (0.25 mm; Camag No. 30179 or Merck Kieselgel 60). Solvent systems were toluene–acetonitrile–acetic acid (65:35:1) (system A) or methanol–chloroform (3:1) (system B). Autoradiography was carried out by using Kodak NS-2T X-ray film.

Mass spectrometry was carried out on a HP5985 (Lilly Research Laboratories, Greenfield, IN) or a AEI MS 50 high-resolution mass spectrometer (Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada) using a direct-probe method for sample introduction.

Design of Laboratory Experiments. A series of culture flasks (125 mL) were prepared containing 10 g (dry weight) of sediment and 100 mL of pond water fortified with fluridone (5.0 μg/mL; 903 dpm/mL). The sediment was collected from farm pond and river bottoms (Table I), stored at 4 °C, and used without drying, within 1 week of collection. Pond water was collected from untreated (control) ponds used in fluridone field experiments. The water had a pH of 7.8; total suspended solids = 19 mg/L; total dissolved solids = 480 mg/L; chlorophyll = 142 μg/L. The sediment–water mixtures in culture flasks were flushed with nitrogen for 1 min, loosely stoppered, and held at 22–25 °C. The flasks were shielded from direct light by placing them in a cardboard container which was open at the top. Several samples of fortified pond water and sediment were autoclaved (30 min) separately. The autoclaved substrates were combined, loosely stoppered and held as described. In addition, sediment–water blanks were prepared. Samples of each sediment were removed (in duplicate) at 3, 6, 9, 16, and 26 months posttreatment. Water and sediment were separated by filtration (Whatman No. 1 paper), and each entire sample was analyzed separately.

Table I. Characteristics of Sediment Used in Laboratory and Field Studies

sediment no.	pH	O.M.,[a] %	clay, %	sand, %	silt, %
1	7.8	3.3	77	0	23
2	7.6	6.4	75	1	24
3	7.7	4.0	48	7	45
pond 1	6.8	5.2	58	5	37
pond 2	7.1	15.8	42	12	36

[a] O.M. = organic matter.

Additional experiments were conducted to study fluridone degradation under aerobic (air) or anaerobic (nitrogen) conditions in sediment–water systems. Respirometer flasks similar in design to those of Simsiman and Chesters (1976) containing 15 g (dry weight) of sediment (sediments 1, 2, or 3, Table I) and 150 mL of pond water were allowed to incubate (25 °C) in a controlled environment room for 14 days. Each flask was attached to a manifold through which air (CO_2-free grade) or nitrogen (prepurified grade; <10 ppm of O_2), both saturated with water, was introduced at 1–2 mL/min. A photoperiod of 8-h darkness and 16-h light was maintained.

Following the incubation period, an acetone solution of either [*carbonyl*-^{14}C]fluridone (experiment 1) or [*N-methyl*-^{14}C]fluridone (experiment 2) was added to each flask to give water concentrations of 5 μg/mL (2500 dpm mL) fluridone. The flasks were attached to two traps in series; the first contained 25 mL of H_2SO_4 (36 N) and the second ethanolamine–ethanol (1:2) (Simsiman and Chesters, 1976). The trapping solutions were changed every 3 months and inspected weekly to ensure that no loss of trapping solution occurred. After 9 months the flasks were removed, and the water and sediment phases were separated by filtration.

Design of Field Experiments. Pond water (similar in characteristics to that used in laboratory experiments) was fortified with a solution of [*N-methyl*-^{14}C]fluridone (1.0 mL) to give concentrations of 5 μg/mL in 900 mL of water. The water was placed in Pyrex flasks which were stoppered and placed on a board covered with black plastic at the field site in sunlight. Several flasks were wrapped with aluminum foil to serve as darkened controls. Flasks were removed after 0.5 h, 1 h, 2 h, 8 h, 12 h, 18 h, 24 h, 3 days, 4 days, and 8 days exposure to sunlight. Dichloromethane (10 mL) was added immediately at each sampling time, the flasks were returned to the laboratory and held at 4 °C, and the extraction was completed within 1 week of collection.

Two small ponds (5 × 3.5 m outer dimensions; 3000-L volume) similar to those described by Muir et al. (1980)

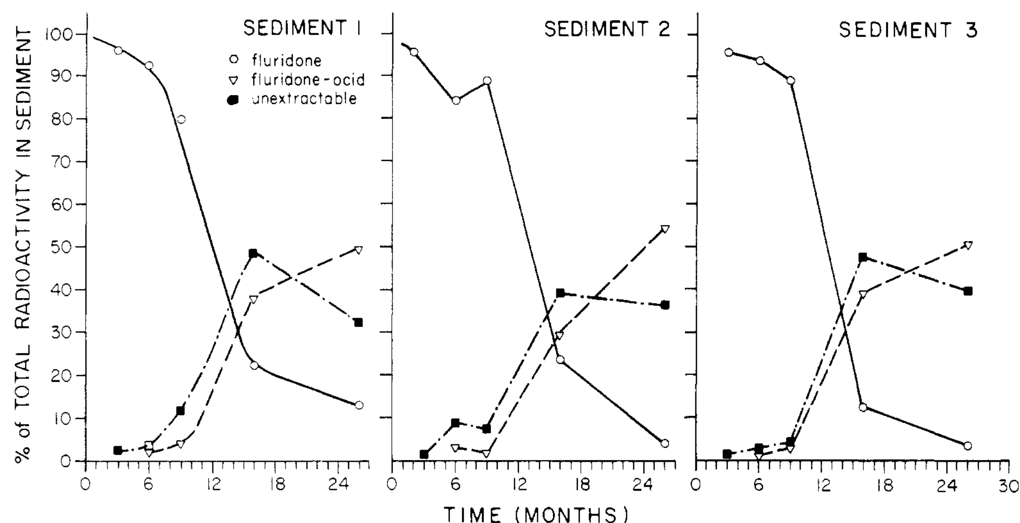

Figure 1. Degradation of [^{14}C]fluridone in three sediments over a 26-month incubation period. "Unextractable" indicates radioactivity not extracted with aqueous acetonitrile.

were each treated at the recommended application rate (100 μg/L) with an aqueous suspension of fluridone (4AS formulation; Elanco Products Canada, Ltd.), which also contained 40 μCi of [*carbonyl*-^{14}C]fluridone. Samples of water (0.9 L) were collected at 0.5, 4, 8, 12, 21, and 24 h, daily for 1 week, once per week for 10 weeks, and then every 2 weeks until 20 weeks posttreatment. Sediment (0–3-cm cores) was collected daily for 1 week following herbicide application and then at the same frequency as water samples. Water and sediment sampling techniques have been described in greater detail previously (Muir et al., 1980).

Water Extraction. Samples of water from laboratory experiments (100–150 mL) were adjusted to pH 2 (in HCl) and extracted by shaking with dichloromethane (3 × 50 mL). Prior to extraction, 1-mL volumes of each water sample were counted by scintillation counting. Water from field experiments (0.9 L) was adjusted to pH 2 and extracted with dichloromethane (150, 75, and 75 mL). Dichloromethane extracts were dried by passing through anhydrous sodium sulfate and evaporated to 1 or 2 mL in a rotary evaporator (40 °C). The samples were transferred to graduated test tubes with methanol and evaporated to 0.1-mL volume. Sample extracts were spotted on two TLC plates which were developd with either solvent system A or B. Following autoradiography, spots containing radioactivity were scraped off into test tubes and suspended in methanol. A small portion of the methanol extract was counted by scintillation counting to determine the radioactivity in each spot.

Sediment Extraction. Samples of sediment (0.5 g) from laboratory and field experiments were combusted to determine total ^{14}C content. The remaining laboratory sample (or 25 g wet weight of sediment from field samples) was refluxed with 150 mL of acetonitrile–water (1:1) for 16 h. The reflux mixture was cooled and filtered (Whatman No. 1 paper). The residue was transferred to a centrifuge bottle (Sorval No. 522) and reextracted by shaking with acetonitrile–water (1:1) for 20 min on a wrist-action shaker. The mixture was centrifuged (1000*g*; 20 min) and the supernatant combined with the filtrate. The acetonitrile was carefully removed by evaporation on a rotary evaporator (50 °C), and the resulting aqueous extract was transferred to a separatory funnel and diluted to 50 mL with water. The extracted sediment was mixed and a portion (0.5 g) combusted to determine unextractable radioactivity.

The aqueous phase as adjusted to pH 2 and extracted with dichloromethane as described previously for water samples. Following extraction, portions (1.0 mL) of the aqueous and dichloromethane phases were counted by scintillation counting. Dichloromethane extracts were spotted on TLC plates to isolate fluridone degradation products.

Extraction of "Bound" Sediment Residues. Extracted sediment was reextracted with 1 N NaOH by using the procedure described by Smith and Muir (1980) and U.S. Environmental Protection Agency (1975) for "bound" residues. Humin (alkali-insoluble material) was recovered and washed with dilute NaOH and water, and a portion was combusted. The entire humic acid fraction of each sediment sample was also combusted. Portions of the fulvic acid fraction (1.0 mL) were assayed directly by scintillation counting. The remaining fulvic acid solution was extracted by shaking with dichloromethane. The dichloromethane extracts were concentrated to a small volume, spotted on TLC plates and chromatographed by using solvent system A.

Cleanup and Confirmation Procedures. TLC spots with identical R_f values were pooled and centrifuged (700*g*) to remove silica gel particles. The samples were concentrated to 0.1–0.5 mL in graduated centrifuge tubes for HPLC analysis.

Aliquots of pooled TLC spots were injected on a C_{18} reverse-phase column, and the column effluent was collected in 0.5- or 1.0-min fractions diluted with PCS–xylene and counted by scintillation counting. Radioactivity associated with fluridone degradation products was located by plotting a histogram of the scintillation counting results on the original HPLC chromatogram. The remaining sample was cleaned up on the same HPLC column by trapping the appropriate fraction of eluant. Samples were evaporated just to dryness and dissolved in methanol for mass spectrometry.

RESULTS

Laboratory Experiments. The degradation of fluridone in sediment–water systems in culture flasks over a 26-month period is shown in Figure 1. Fluridone had an average half-life ($t_{1/2}$) of 12 months in the three sediment types that were used. After 26 months less than 13% of the radioactivity in the sediment was in the form of fluridone and 32–40% was unextractable with acetonitrile–water (16-h reflux). Autoclaved sediments extracted after

Table II. R_f Values and HPLC Retention Times for Fluridone and Degradation Products

compound	R_f in each solvent system[a] A	B	HPLC RT,[b] min
I	0.07	0.0	10.0
II	0.2	0.43	11.6[c]
III	0.25	0.73	7.8
IV	0.28	d	5.4
fluridone	0.50	0.73	14.4
V	0.57	0.77	10.8

[a] A = toluene-acetonitrile-acetic acid (65:35:1). B = methanol-chloroform (3:1). [b] Retention time on Ultrasphere ODS by using methanol-water-formic acid (60:40:0.2). [c] Broad peak. All other peaks are symmetrical. [d] Not determined.

Table III. Percent of Total Radioactivity as Minor Degradation Products in Sediment from Culture Flasks Incubated 3-26 Months

time, months	sediment no.	compound, % III	V	immobile[a]
3	1	1.0	1.0	b
	2	1.0	1.0	b
	3	1.0	1.0	b
6	1	1.0	0.5	0.8
	2	1.8	1.1	2.8
	3	1.4	0.9	0.7
9	1	2.5	1.3	1.0
	2	2.0	1.8	0.5
	3	2.1	1.9	0.8
16	1	0.5	b	2.0
	2	0.6	b	0.5
	3	b	b	0.5
26	1	1.6	b	2.4
	2	1.7	b	1.6
	3	1.6	b	2.7

[a] Radioactivity remaining at the origin (system A). [b] Not detected (<0.5%).

Table IV. Distribution of Unextractable Fluridone Residues in Sediments from Culture Flasks Incubated 16 and 26 Months

time, months	sediment	% of ^{14}C in each fraction[a] humin	humic	fulvic	CH_2Cl_2 extract,[b] %
16	1	20.5	1.8	81.9	78.4
	2	14.3	3.9	69.2	76.5
	3	10.4	3.4	77.6	82.8
26	1	27.4	2.2	76.6	71.5
	2	24.8	4.1	60.1	67.4
	3	19.3	6.4	68.0	63.6

[a] Total ^{14}C in combusted residue = 100%. [b] Dichloromethane extraction of the fulfic acid fraction.

Table V. Distribution of Radioactivity following a 9-Month Incubation of Fluridone in the Sediment-Water System under Aerobic (A) and Anaerobic (AN) Conditions

sediment no.	condition	% in each phase[a]: sediment, extracted	sediment, unextracted	water	ethanolamine trap
		(A) [*carbonyl*-^{14}C]Fluridone			
1	A	51.2	9.6	26.1	0.4
	AN	49.6	10.1	41.6	0.2
2	A	64.6	26.0	19.1	0.3
	AN	72.4	4.9	29.9	0.6
3	A	60.2	19.7	27.1	0.3
	AN	54.0	1.8	44.5	0.1
		(B) [*N-methyl*-^{14}C]Fluridone			
1	A	58.8	2.9	47.4	0.5
	AN	31.7	2.3	62.6	0.2
2	A	54.0	16.9	14.6	0.3
	AN	70.5	4.3	38.2	0.2
3	A	76.1	5.3	2.1	0.3
	AN	33.1	1.5	46.5	0.1

[a] Extracted = extracted into dichloromethane. Unextracted = determined by combustion of the extracted sediment. Water = radioactivity extracted into dichloromethane.

26 months of incubation had almost all the radioactivty in the form of fluridone and less than 5% unextractable with aqueous acetonitrile. The lack of degradation of fluridone in autoclaved sediments suggests that biological action is essential for degradation of the herbicide.

Four degradation products of fluridone were isolated by TLC of sediment extracts with solvent systems A and B (Table II). System A gave better resolution of the four compounds, especialy II and V, and was used exclusively for TLC of samples from field studies. Compounds II and III were identified as fluridone–acid and 4-hydroxyfluridone, respectively, based on similar TLC R_f values with analytical standards. Additional confirmation was provided by comparison of retention times of unknowns with analytical standards on HPLC and by mass spectrometry of methylated derivatives of the compounds. Fluridone–acid (II) was the major degradation product of fluridone in sediment extracts, representing 48–54% of total radioactivity at 26 months (Figure 1). The 4-hydroxy derivative (III) was detected at low levels in all sediment extracts with maximum concentration at 9 months (2.5%; sediment 1) (Table III). II had previously been shown to be the major degradation product of fluridone in terrestrial soils (Rainey, 1980). III is a degradation product of fluridone in rats and fish (Rainey, 1980) but has not been reported previously in soil or sediment.

Compound V, which was found only in 3-, 6-, and 9-month samples at low (1.0–1.9%) levels, appeared to be nonphenolic based on its R_f value (Table II). However, mass spectra of the compound showed a molecular ion at m/e 345 and a major fragment at m/e 328, indicating that it was a hydroxylated fluridone derivative. The compound had identical HPLC retention time and TLC R_f (system A) value to those of 2-hydroxyfluridone which had previously been detected as a minor fluridone metabolite in rats and fish (Rainey, 1980). Compound V was therefore assumed to be 2-hydroxyfluridone though the possibility that was the 3- or 5-hydroxy derivative could not be completely ruled out. The apparent low polarity of V compared to III may be due to the possibility of intramolecular hydrogen bonding of the hydroxy at the 2 position with the carbonyl group on the pyridinone ring. Another compound (IV) was detected near III on TLC–autoradiograms in sediment extracts, but only at trace levels (<0.5%) in 6- and 9-month samples, and could not be identified.

Results of the alkaline extraction of extracted sediments from 16- and 26-month samples are given in Table IV. A major portion of the radioactivity was found in the fulvic fraction and most was extractable into dichloromethane. TLC and mass spectral analysis of the extracts indicated that the fulvic fraction was entirely in the form of II at both sampling times. A slightly larger percentage of the radioactivity was unextractable with 1 N NaOH in 26-month sediments compared to 16-month sediments, suggesting further breakdown of the fluridone molecule.

Results of the incubation of [*carbonyl*-^{14}C]- or [*N-methyl*-^{14}C]fluridone in sediment–water respirometer systems having air or nitrogen flow are given in Table V. Most of the radioactivity was associated with the sedi-

Table VI. Degradation of Fluridone in Pond Water in Stoppered Pyrex Flasks under Sunlight: Distribution of Radioactivity Extracted with Dichloromethane

time, h	I	II	III	fluridone	V	unextractable[b]
	% of total radioactivity added					
0.5	*a*	*a*	*a*	99.2	0.8	*a*
4.0	*a*	*a*	0.9	94.6	1.2	*a*
12.0	*a*	*a*	0.7	97.8	0.7	*a*
24.0	*a*	*a*	0.9	97.6	0.8	*a*
48.0	*a*	*a*	0.2	83.7	1.2	*a*
72.0	1.0	0.9	3.9	88.7	2.8	*a*
96.0	1.2	0.8	4.8	70.5	1.6	17.0
192.0	3.2	2.2	8.3	23.4	2.0	56.2

[a] Not detected (<0.2%). [b] Difference between radioactivity added and dichloromethane-extractable products.

ments, especially in the flasks held under aerobic conditions, which was expected from the strong adsorption and low percent desorption of the herbicide in sediments (Muir et. al., 1980). From 70 to 104% of the carbonyl-^{14}C label and from 62 to 112% of the *N*-methyl-^{14}C label were extractable from sediments wiith aqueous acetonitrile. The higher percent unextractable in the aerobic sediments may reflect greater rates of degradation of fluridone to II (which is only partially extracted with aqueous acetonitrile) under aerobic conditions. Losses of radioactivity via volatilization and trapping in ethanolamine represented less than 1% of the radioactivity of either ^{14}C-labeled compound that was added to the incubation flasks. As expected, losses of $^{14}CO_2$ were slightly higher under aerobic conditions. The identity of the radioactivity in the acid and ethanolamine traps was not determined.

Field Studies. Exposure of [*N-methyl*-^{14}C]fluridone in pond water to sunlight in a stoppered Pyrex flask over an 8-day period yielded a number of degradation products. Compounds II, III and V, previously identified in sediment from laboratory studies, were detected; the proportion of radioactivity represented by each compound is shown in Table VI. Compound I was identified as desphenylfluridone by comparison of its mass spectrum and HPLC retention time (Table II) with those of an authentic standard of the compound. The mass spectrum of I had an intense molecular ion at *m/e* 253 and a base peak at m/*e* 252, which are characteristic of the desphenyl compound. The corresponding des[(trifluoromethyl)phenyl]fluridone which had similar mobility on TLC plates in systems A and B was not detected. Several other minor degradation products were also detected by TLC–autoradiography but could not be identified.

Fluridone had a $t_{1/2}$ of about 6 days in the flasks in sunlight, compared to an average of 5 days observed in previous studies with nonradiolabeled compounds in outdoor ponds (West et al., 1979; Muir et al., 1980). Degradation of the herbicide in darkened, aluminum foil wrapped flasks was negligible (<5%) over the 8-day period which indicated that photolysis was the major degradative pathway under the conditions of the experiment. On the first day of the experiment, sunlight intensity measured 4 cm above the surface of a nearby pond was 1450 microeinsteins/m^{2}s (at 1200 h). The lower rate of disappearance of fluridone in the present study compared to a previously reported $t_{1/2}$ of 23 h (West et al., 1979) may be due to the use of Pyrex glass and to the use of outdoor conditions.

After 8 days of exposure, only 44% of the added radioactivity was extractable from the water (at pH 2) with dichloromethane (Table VI). Comparison of extractable radioactivity with total ^{14}C (determined by direct counting of small volumes of water) indicated that no radioactivity was lost by volatilization from the stoppered flasks. Thus considerable transformation of fluridone to polar unextractable products occurred.

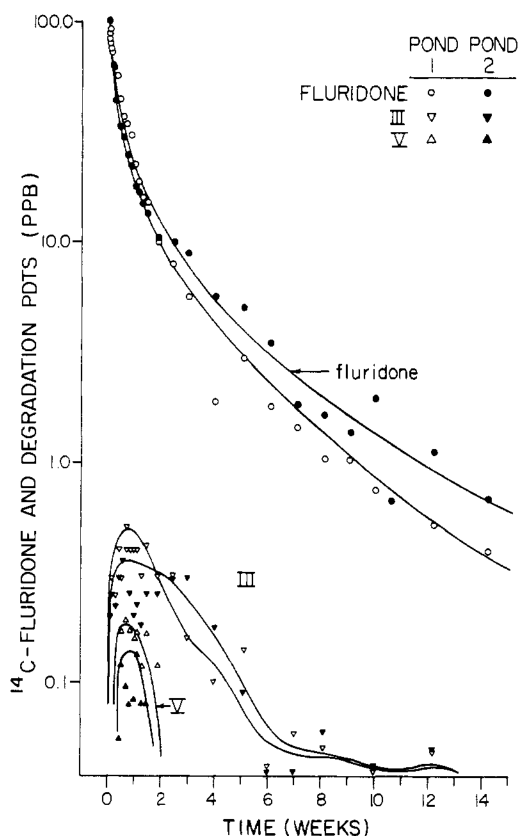

Figure 2. Disappearance of [^{14}C]fluridone in pond water following application at the rate of 100 μg/L to two small ponds. III = 4-hydroxyfluridone; V = 2-hydroxyfluridone.

Results of the analyses of pond water samples following treatment of two small ponds with [*carbonyl*-^{14}C]fluridone are shown in Figure 2. Compounds III and V were detected in sample extracts by TLC–autoradiography at low levels (<0.5 μg/L). The identity of both compounds was confirmed by pooling samples of identical R_f values and chromatographing on a reverse-phase HPLC column. The elution of radioactivity at the expected HPLC retention time of III and V, together with the isolation by TLC, was taken as positive identification as illustrated in Figure 3. Other photolysis products that had been identified in flask studies were not detected.

Both III and V showed maximum concentrations in pond water at 5 days posttreatment. Levels of V declined rapidly, and the compound was undetectable (<0.05 μg/L) after 2 weeks. III showed greater persistence than V and was detectable for about 12 weeks posttreatment at trace (0.05 μg/L) levels. The degradation products were not present in anywhere near the same proportion of total radioactivity as that found in studies with [*N-methyl*-^{14}C]fluridone in flasks in sunlight (Table VI). Fluridone remained the major residue in the water column of both ponds with a $t_{1/2}$ of 2–3.5 days (ponds 1 and 2, respectively).

Fluridone levels in the pond bottom sediment were monitored for 20 weeks posttreatment by combustion of small portions of the unextracted sediment and by extraction with aqueous acetonitrile. TLC–autoradiography of pond sediment extracts did not reveal any degradation products of fluridone (detection limit 0.05 μg/g). This may

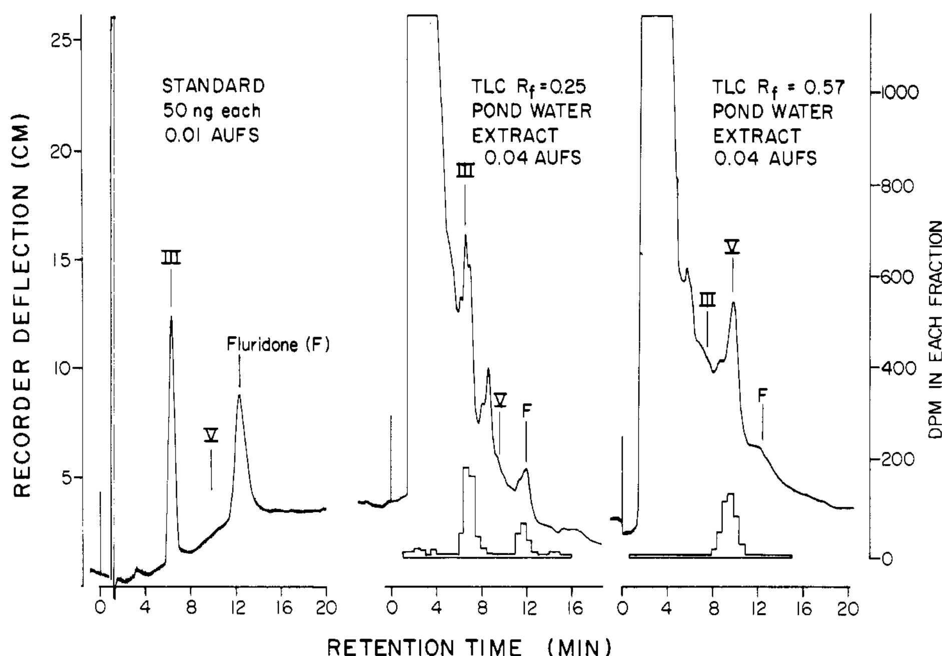

Figure 3. HPLC chromatograms of analytical standards (50 ng) of 4-hydroxyfluridone (III) and fluridone (F) and pond water extracts previously chromatographed on TLC plates (system A). Areas on the TLC plates corresponding to R_f 0.25 and R_f 0.57 were injected, and radioactivity, indicated by histograms below each chromatogram, was measured by scintillation counting.

Table VII. Percent of [^{14}C]Fluridone in the Sediment or Water Compartments of Ponds 1 and 2

time	pond no.	% of fluridone accounted for[a] sediment	water	total
1 day	1	14.1	76.7	90.8
	2	33.5	67.4	100.9
4 days	1	24.8	33.5	58.3
	2	23.1	32.3	55.4
10 days	1	30.1	17.7	47.8
	2	52.5	16.1	68.7
3 weeks	1	44.8	6.3	51.1
	2	62.5	9.8	73.3
7 weeks	1	23.4	1.6	25.0
	2	44.4	1.6	46.0
20 weeks	1	18.1	0.3	18.4
	2	34.2	0.3	34.5

[a] Where 282 mg of fluridone was added. Water volume volume = 3000 L in both ponds. Sediment weight was 250 kg (0-3-cm depth; density 0.8 g/cm^3).

have been due to the small quantity of sediment that was extracted (10 g dry weight) and the relatively low concentration of fluridone (0.2–1.0 μg/g dry weight).

A mass balance of fluridone in ponds 1 and 2 was calculated by using estimates of pond water volume (3000 L) and sediment (0–3-cm depth) (Table VII). Ponds 1 and 2 showed maximum quantities of fluridone in sediment at 3 weeks posttreatment. After 20 weeks the percent of fluridone residue in the sediment was less than 50% of the maximum observed, which indicated a $t_{1/2}$ in sediment of about 17 weeks. In pond 1, 50% of the radioactivity that was added could not be accounted for after 3 weeks. In pond 2, fluridone radioactivity had a $t_{1/2}$ of about 7 weeks. The differences in the persistence of fluridone between the two ponds may be due to the higher organic matter content of sediments in pond 2 compared to those in pond 1 (Table I).

DISCUSSION

The results of the present work indicate that extensive photodegradation of the fluridone molecule to unextractable polar products was the major degradation pathway of the herbicide in aquatic systems under actual-use conditions. Ring hydroxylation occurred in light-restricted sediment water systems, in pond water in flasks exposed to sunlight, and in outdoor ponds. However, the photochemical oxidation reactions were much more rapid ($t_{1/2}$ of 6 days for fluridone in flasks in sunlight) than microbial oxidation in sediment ($t_{1/2}$ of 12 months in culture flasks). There were striking differences between the types and proportions of degradation products observed in the field and laboratory studies. The proportions of III and V were much lower under field conditions than in flasks in sunlight. Fluridone–acid (II) was not detected in water or sediment from field studies which was surprising in view of the predominance of this compound in sediment and water in culture flask incubations in the laboratory. Both I and II were detected as minor products after a 72-h exposure to sunlight in flasks (after the appearance of compounds III and V) which suggested that II and I were formed following ring hydroxylation. The desphenyl compound can be determined directly by GLC (using a nitrogen-specific detector), but examination of dichloromethane extracts of water from ponds treated at 0.7 and 5.0 mg/L (Muir et al., 1980) did not show detectable levels of I (detection limit 0.1 μg/L). Methylation (diazomethane) of the same extracts and GLC analysis did not reveal the presence of II.

Another possible pathway of loss of degradation products and fluridone from pond water under field conditions may be via volatilization. Henry's constants for fluridone and I were calculated from the rate of loss of the compounds from a column of distilled water sparged continuously with nitrogen as described by Mackay et al. (1979) (Table VIII). Half-lives of fluridone and I in the ponds (0.5-m depth; 10-m^2 surface area) were calculated by use of the equation of Mackay and Leinonen (1975). The volatilization half-lives of fluridone and I were 50.8 and 4.2 days, respectively. These calculations suggest that volatilization of fluridone was not a major factor in the disappearance of the herbicide from the ponds but they may help to explain the inability to detect I under field conditions. The actual rate of loss of the herbicide and its volatile degradation products would be expected to be

Table VIII. Volatilization Half-Lives of Fluridone and Desphenylfluridone in Outdoor Ponds Using Equations of Mackay and Leinonen (1975)

compound	H, atm m³ mol[a]	$1/(KL)$, m/h[b]	$t_{1/2}$, days
fluridone	1.0×10^{-6}	3537.4	50.8
desphenylfluridone	1.1×10^{-5}	290.5	4.2

[a] Henry's constant calculated as described by Mackay et al. (1979). [b] $1/(KL) = 1/k_L + RT/(Hk_G)$ (m/h⁻¹), where k_L = liquid transfer coefficient (m/h) and k_G = gas transfer coefficient (m/h), R = gas constant [m³ atm/(mol K)], and T = K (298). For fluridone, k_L = 0.073 and k_G = 7.017 m/h; for desphenylfluridone, k_L = 0.0834 and k_G = 8.002 based on published values for H_2O and CO_2 transfer (Mackay et al., 1979).

reduced somewhat by the proportion of fluridone sorbed to bottom sediments, aquatic plants, and seston (Mackay et al., 1979).

The persistence of [*carbonyl*-^{14}C]fluridone in pond sediment was much shorter than that observed in sediment–water systems in the laboratory. Previous work with unlabeled fluridone (Muir et al., 1980) indicated half-lives of greater than 12 months under field conditions which was similar to that observed in laboratory studies. The timing of the herbicide application to the artificial ponds may be an important factor in explaining the differences between the field experiments. Previous field applications were made in mid July while the experiments with radiolabeled fluridone were started in mid-June which allowed an additional 4–6 weeks of high water temperatures and long daylight hours. The $t_{1/2}$ in water of fluridone applied in mid-June was about half of that observed in the mid-July treatment (3.5 vs. 7.0 days). While fluridone appeared to be quite persistent in light-restricted culture flask experiments, the susceptibility of the herbicide to photodegradation permitted relatively rapid disappearance under actual field conditions.

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Determination of Urinary Residue Levels of the N-Dealkyl Metabolites of Triazine Herbicides

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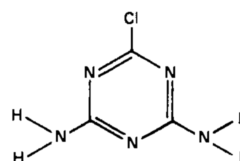
Analytical methodology for monitoring human exposure to the triazine herbicides atrazine, simazine, and propazine is needed because of the widespread use of these compounds in agriculture. In animals, the chief urinary metabolites that have been reported are the corresponding N-dealkyl triazines 2-chloro-4-amino-6-(ethylamino)-*s*-triazine (I) and 2-chloro-4-amino-6-(isopropylamino)-*s*-triazine (II). An analytical procedure for determining urinary levels of these metabolites by gas chromatography is presented. A third metabolite, 2-chloro-4,6-diamino-*s*-triazine (III), found in the urine of rats dosed with the three parent compounds, is also reported here. This compound appears to be the major metabolite in rats.

A number of nitrogenous compounds find their way into the environment, among which are the triazine herbicides. Although the toxicity of these compounds to mammals is generally low (2000–5000 mg/kg oral LD_{50} in rats), they are used in such large quantities that indirect analytical methodology is needed to assess exposure of agricultural workers. The metabolism of the triazine herbicides appears to be rather complicated, but various researchers have reported that the major animal metabolites arise from N-dealkylation to produce compounds with the general structure



(Bakke et al., 1967, 1971, 1972; Böhme and Bär, 1967; Robbins et al., 1968; Hutson et al., 1970; Larson et al., 1971; Crayford and Hutson, 1972; Dauterman and Muecke, 1974; Larsen and Bakke, 1975). The general indication is that

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