

# Fluridone and N-methylformamide Residue Determinations in Ponds

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## ABSTRACT

NMF (N-methylformamide), a laboratory photolytic product of fluridone (1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4(1H)-pyridinone), was monitored in conjunction with fluridone in two, 0.014 ha hydrilla infested ponds in a 167 day study. NMF is a known teratogen, fetotoxin, hepatotoxin and cytotoxin. Previous to this study, NMF had not been monitored in a natural environment following a herbicidal application of fluridone. Pond 1 was treated with 150 ppb fluridone in December, 1987; while Pond 2 received 466 ppb fluridone in October, 1987. Of the 59 water samples analyzed for NMF and fluridone, no NMF was found at a detection limit of 2 ppb. Fluridone residuals ranged from 677 to 1 ppb; they diminished to less than 1 ppb after 167 days.

*Key words:* NMF, hydrilla, chlorophyll<sub>a</sub>, herbicides, degradation, water quality.

## INTRODUCTION

Fluridone (1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4(1H)-pyridinone), produced by the Elanco Products Company, a Division of Eli Lilly and Co., was registered in 1986 for aquatic use and controls a variety of submersed and emersed aquatic plants. Fluridone was reported in 1983 to undergo photolysis under laboratory conditions to produce several photolytic products; one of the principle components was NMF (N-methylformamide) (Saunders and Mosier 1983). However, NMF was not observed in field studies conducted outdoors in artificial ponds with radiolabelled fluridone (Berard and Rainey 1981). In a recent review of the herbicide prior to its use for weed control in a small New York State lake, health concerns were expressed over the potential of NMF being formed in natural environments after the application of fluridone (Williman 1987). These concerns were based upon the knowledge that NMF, which can permeate human skin, is a known teratogen, fetotoxin, hepatotoxin and cytotoxin (Stula and Krauss 1977, Barlow and Sullivan 1982, Whitby *et al.* 1982 Kennedy 1986). Since NMF has not been subjected to an evaluation in a natural environ-

ment after an application of an actual fluridone formulated product, this study was designed with that objective.

## METHODS AND MATERIALS

Two 0.014 ha experimental ponds, constructed on the campus of the University of Central Florida, Orlando, Florida in 1975, were utilized in this study. The ponds, designed for aquatic experimentation, are 18.3 m x 76.2 m; their sides are sloped along a 1:4 grade to provide a longitudinal depth gradient of 0.31 to 1.83 m. The ponds, which can be drained and refilled from a 122 m deep well, have their water level periodically adjusted with well water by movements of a float valve. The water depth within the ponds did not vary more than 5 cm throughout this study. The hydrosol of the ponds is sand underlying a thin layer of sapropel. With the exception of chelated copper, aquatic herbicides had not been applied within these water bodies. Prior to this study, the ponds were void of aquatic vegetation; on May 1, 1987 they were planted with hydrilla (*Hydrilla verticillata* Royle) by spreading 30 kg of the plant, cut into fragments, over their surfaces. The ponds remained undisturbed until October 7, 1987, at which time the mean hydrilla biomass was determined.

Fluridone (formulated as a 41.7% fluridone aqueous suspension) was applied to Pond 1 on December 5, 1987 and to Pond 2 on October 10 and 17, 1987, (split treatment) by surface application. For each application, the herbicide was added to 95 L water along with 250 mg/L polymer sinking agent (Polycontrol II), mechanically agitated, and spread over the surface by spray handgun utilizing 180 psi produced by a 6 gpm diaphragm pump. An airboat was utilized to minimize turbidity and disturbance of the hydrilla. Pond 1 was treated with fluridone at the rate of 150 ppb a.i., while two treatments of 233 ppb each were applied to Pond 2 for a total of 466 ppb a.i. The maximum label rate within a period of one year for fluridone, in ponds 0.9-1.3 m deep, is 107 ppb a.i. at a depth of 0.9 m (EPA Registration No. 1471-127). This label rate was exceeded in this study to achieve a concentration of fluridone in Pond 1 at the maximum acceptable residue level allowed in potable water (150 ppb a.i.; Federal Register 1987). The treatment rate for fluridone in Pond 2 was 4.5 times the EPA labeled fluridone concentration allowed for pond treatments.

Water samples, analyzed for residual fluridone and NMF, were collected at 25 cm below the surface in new, precleaned 1 L amber glass bottles at two fixed stations; shallow (0.5 m) and deep (1.5 m). The samples were collected prior to treatment (pretreatment), immediately after treatment (post-treatment), 6 hrs, 4 days, weekly for 5 weeks, biweekly for an additional 6 weeks and finally

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monthly for 3 months following treatments. Sampling was conducted over 161 days in Pond 1 (December 5, 1987-May 14, 1988) and over 167 days in Pond 2 (October 10, 1987-April 23, 1988). Post-treatment and 6 hr (after treatment) samples were not taken for the first split treatment in Pond 2; in addition 3 sample bottles were broken during shipment. In total, 59 samples were analyzed for fluridone and NMF concentrations. Immediately after the collections were taken, the samples were refrigerated at 4 C until shipped in styrofoam chests by overnight delivery to the analytical laboratory in Greenfield, Indiana. The samples, in turn, were chilled under refrigeration at the analytical laboratory until the residual analyses were performed.

Three stations (shallow, 0.5 m; mid-depth, 1.0 m, deep, 1-5 m) were sampled in the ponds on a monthly basis for physicochemical determinations (October, 1987-May, 1988). Water samples for these determinations were collected with a 60 L/min submersible bilge pump at mid-depth within the water column at each station. Samples were stored in 1 L opaque Nalgene bottles for transport; chemical determinations were performed within 3 hrs of collection. Dissolved oxygen samples were 'fixed' in the field with manganese sulfate, alkalinity-iodide-sodium azide and concentrated sulfuric acid. Specific conductivity and water temperature were measured in the field with a Model CTU-4 Montedoro-Whitney S-C-T meter. Hydrilla biomass was determined on October 7, 1987 and May 28, 1988 (8 random stations per pond) using the Osborne aquatic plant biomass sampler (APHA 1985). Fresh weight biomass was determined from washed plant samples after they had been spun at 560 rpm to remove excess water.

Water samples were analyzed for fluridone and NMF using water from the same sample. The water was passed through a C18 Sep-Pak cartridge to retain the fluridone, while NMF passed unretained through the cartridge with the water. The fluridone was eluted from the cartridge with methanol, concentrated, and its amount determined with reverse-phase high-performance liquid chromatography at a detection limit of 1 ppb (West and Turner 1988). The NMF was concentrated by evaporation of the water samples with final determinations completed by gas chromatography using a Hall electrolytic conductivity detector at a detection limit of 2 ppb (West and Turner 1988).

Hydrogen-ion concentration and total alkalinity were determined using a Corning Model 125 pH meter with digital readout to the nearest 0.01 pH unit. Total alkalinity, turbidity, tannin, and water color were determined using methods described in APHA (1985). Tannin and water color were determined from filtered water samples (mesh size = 0.45  $\mu$ ). Ammonia ( $\text{NH}_3$ ; Nesslerization method), nitrite nitrogen ( $\text{NO}_2\text{-N}$ ; sulfanilamide method), nitrate nitrogen ( $\text{NO}_3\text{-N}$ ; hydrazine sulfate method) orthophosphate ( $\text{O-PO}_4$ ; ammonium molybdate-ascorbic acid method) and dissolved organic phosphorus (digestion with persulfate) were also determined from filtered water samples using colorimetric methods described in APHA (1985) and EPA (1974). Chlorophyll<sub>a</sub>, an estimate of algal biomass, was determined colorimetrically using the method of Richards with Thompson (1952). One liter water samples were filtered (glass fiber filter mesh size = 0.45  $\mu$ ) for this determination. The pigment was extracted

with 10 ml of 90% acetone while being stored in the dark at 4 C for 24 hr. Colorimetric methods were performed using a Beckman Model 26 spectrophotometer with micro-cuvette.

## RESULTS AND DISCUSSION

The hydrilla biomass in Pond 1 at the start of the study ( $1.81 \pm 0.34 \text{ kg/m}^2$  fresh wt.) was not significantly different ( $P \leq 0.05$ ) than the biomass in Pond 2 ( $1.91 \pm 0.76 \text{ kg/m}^2$  fresh wt.). At that time, hydrilla was rooted in nearly 90% of the sediment and had become matted at the surface throughout much of the ponds. By the end of the study in May, 1988, the biomass of the hydrilla had increased 65% in Pond 1 ( $2.96 \pm 0.94 \text{ kg/m}^2$  fresh wt.). The hydrilla biomass in Pond 2, which had received 4.5 times the EPA labeled rate of fluridone for ponds, decreased 46% ( $1.03 \pm 0.49 \text{ kg/m}^2$  fresh wt.). While the hydrilla in both ponds had apparent herbicidal effects (chlorosis) after treatment and for nearly 3 months thereafter, these symptoms were not evident in May. The absence of hydrilla control within the ponds was probably due to the late autumn treatments, i.e. fluridone applied during plant senescence resulting in limited herbicide uptake. This could have accounted for the herbicidal symptoms without plant death. Chlorophyll<sub>a</sub> values, an indirect measure of algal biomass ranged from 17.7 to 2.2  $\mu\text{g/L}$  in Pond 1 and between 12.5 and 3.7  $\mu\text{g/L}$  in Pond 2, Table 1. At the beginning of the study no significant differences ( $P \leq 0.05$ ) were observed between Pond 1 and 2 for any of the physicochemical measurements, Table 1. Water clarity was good in both ponds and allowed light penetration to the bottom (Secchi disc transparency). Water color declined during the study and varied between 11.7 and 28.3 Pt-Co units in Pond 1 and between 9.5 and 21.0 Pt-Co units in Pond 2. An increase in water color is generally associated with a decline in hydrilla biomass following control (Schmitz and Osborne 1984); however, this relationship did not occur in our study. Values for tannin concentration, the causal agent for water color, were similar between ponds and had declining trends, Table 1. Mean dissolved oxygen values ranged from 4.2 to 9.0 ppm in Pond 1 and from 4.2 to 10.1 ppm in Pond 2. Total alkalinity, while similar between ponds in October, 1987, tended toward higher values in Pond 2 as compared to Pond 1 after the application of fluridone. Mean total alkalinity reached 141 mg/L  $\text{CaCO}_3$  in January 1988 in Pond 1 and 109 mg/L  $\text{CaCO}_3$  in February, 1988 in Pond 2. These mid-study peaks were probably due to low photosynthetic activity within the ponds during mid-winter, Table 1. Nitrite nitrogen was not detected in either pond. Nitrate nitrogen, which increased within the ponds after the herbicide applications, reached maximum mean values of 68 ppb and 52 ppb in April, 1988 in Ponds 1 and 2, respectively. Little change was noted for orthophosphate concentrations (1-7 ppb, Pond 1; ND-7 ppb; Pond 2). On the other hand, dissolved organic phosphorus concentrations increased progressively throughout the study to reach highs in early spring; values ranged from 12 to 143 ppb in Pond 1 and 14 to 169 ppb in Pond 2, Table 1.

No adverse environmental conditions which might alter the natural photolytic degradation of the fluridone were

TABLE 1. MONTHLY MEAN PHYSICOCHEMICAL MEASUREMENTS IN EXPERIMENTAL POND 1 AND 2, OCTOBER, 1987-MAY, 1988. THE STANDARD ERRORS OF THE MEANS ARE PRESENTED IN PARENTHESES.

Parameter	Experimental Pond 1							
	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
Water temperature (C)	24.0 (0.8)	19.1 (0.1)	20.2 (0.1)	15.5 (0.2)	14.2 (0.2)	17.6 (0.1)	22.2 (0.1)	25.5 (0.1)
Water color (Pt-Co units)	22.0 (0.8)	28.3 (0.9)	23.0 (1.7)	23.3 (2.7)	18.3 (0.3)	14.3 (0.3)	14.0 (0.5)	11.7 (0.5)
Turbidity (NTU)	3.7 (0.9)	3.3 (0.8)	1.3 (0.2)	1.1 (0.1)	1.1 (0.2)	1.5 (0.1)	1.7 (0.2)	2.1 (0.2)
Tannin (ppb)	22 (0)	24 (0)	17 (0)	27 (0)	26 (0)	10 (0)	25 (0)	21 (0)
Dissolved oxygen (ppm)	5.0 (0.4)	6.9 (0.1)	9.0 (0.5)	5.6 (0.5)	7.7 (0.4)	6.5 (0.5)	6.5 (0.2)	4.2 (0.2)
pH	7.6 (0.1)	8.3 (0.0)	7.8 (0.0)	7.0 (0.1)	7.5 (0.1)	7.2 (0.1)	7.4 (0.0)	7.1 (0.1)
Total alkalinity (mg/L CaCO <sub>3</sub> )	74 (5)	62 (4)	67 (8)	106 (2)	109 (4)	99 (3)	109 (2)	106 (3)
Specific conductivity (µmhos/cm @ 25 C)	215 (7)	168 (14)	194 (28)	271 (4)	270 (7)	265 (11)	310 (2)	317 (4)
Ammonia (ppm)	.05 (.02)	.17 (.03)	.43 (.07)	.17 (.01)	1.88 (.42)	.70 (.09)	.25 (.04)	1.13 (.05)
Nitrite nitrogen (ppb)	ND	ND	ND	ND	ND	ND	ND	ND
Nitrate nitrogen (ppb)	10 (0)	7 (0)	6 (0)	3 (0)	ND	21 (0)	68 (0)	33 (3)
Orthophosphate (ppb)	3 (0)	2 (0)	4 (0)	5 (0)	7 (0)	6 (0)	ND (1)	4
Dissolved organic phosphorus (ppb)	23 (10)	47 (20)	12 (0)	59 (20)	179 (10)	50 (20)	143 (30)	107 (10)
Chlorophyll <sub>a</sub> (µg/L)	15.8 (2.6)	14.0 (2.1)	2.2 (0.1)	7.7 (2.9)	6.8 (1.2)	5.1 (0.3)	3.6 (0.5)	17.7 (1.8)
Parameter	Experimental Pond 2							
	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
Water temperature (C)	24.2 (0.1)	20.0 (0.1)	20.5 (0)	15.9 (0.2)	15.3 (0.4)	17.8 (0.1)	22.7 (0.1)	26.2 (0.1)
Water color (Pt-Co units)	21.0 (0.5)	19.3 (0.3)	18.7 (0.3)	20.0 (0.0)	15.0 (0.0)	10.3 (0.3)	10.8 (0.3)	9.5 (0.0)
Turbidity (NTU)	1.8 (0.3)	1.3 (0.1)	1.2 (0.1)	1.0 (0.2)	1.0 (0.1)	1.2 (0.1)	2.0 (0.3)	2.3 (0.1)
Tannin (ppb)	24 (0)	24 (0)	20 (0)	28 (0)	25 (0)	14 (0)	22 (0)	19 (0)
Dissolved oxygen (ppm)	4.2 (0.4)	7.4 (0.4)	7.7 (0.3)	5.7 (0.3)	8.6 (0.3)	8.7 (0.3)	10.1 (0.3)	8.6 (0.2)
pH	7.6 (0.1)	7.6 (0.1)	7.5 (0.1)	7.0 (0.1)	7.8 (0.1)	8.0 (0.1)	8.0 (0.0)	8.5 (0.1)
Total alkalinity (mg/L CaO <sub>3</sub> )	80 (4)	105 (3)	122 (2)	141 (1)	129 (3)	104 (1)	102 (1)	95 (3)
Specific conductivity (µmhos/cm @ 25 C)	225 (9)	277 (7)	328 (3)	349 (4)	311 (1)	275 (3)	307 (7)	285 (8)
Ammonia (ppm)	.03 (.01)	.22 (.05)	.45 (.02)	1.70 (.70)	2.32 (.23)	.38 (.06)	.40 (.05)	.15 (.07)
Nitrite nitrogen (ppb)	ND	ND	ND	ND	ND	ND	ND	ND

Nitrate nitrogen (ppb)	14 (0)	6 (0)	8 (0)	4 (0)	10 (0)	22 (0)	52 (0)	26 (2)
Orthophosphate (ppb)	4 (0)	2 (0)	4 (0)	4 (0)	7 (0)	5 (0)	1 (0)	2 (0)
Dissolved organic phosphorus (ppb)	14 (0)	23 (10)	10 (0)	53 (10)	199 (40)	169 (30)	139 (10)	139 (33)
Chlorophyll <sub>a</sub> (µg/L)	8.2 (1.4)	7.6 (2.7)	5.2 (0.5)	3.9 (0.2)	3.7 (0.4)	3.8 (0.7)	4.4 (0.4)	12.5 (2.7)

ND—not detected at 1 ppb

noted within the ponds during this field study, the clarity of the water allowed ample incoming radiation for the photolytic decomposition of the fluridone, even during dense hydrilla biomass. Secchi disc transparency extended to the bottom in both ponds throughout the duration of the study. The ponds are classified as mesotrophic, based upon nutrient concentrations (Canfield and Hodgson 1983) and are considered representative of many Florida aquatic environments (Shannon and Brezonik 1972, Beaver *et al.* 1981 Small *et al.* 1985).

Of the 59 water samples collected from Ponds 1 and 2 for NMF analysis, none was not detected in any of the samples at or above the detection limit of 2 ppb. These NMF measurements were made from water samples containing between 1 and 677 ppb fluridone over a sampling period of 167 days (24 weeks). The labeled concentration for fluridone allowed for potable water is 150 ppb a.i. Since NMF was not found in our samples, especially those taken from the pond treated with 4.5 times the EPA allowable concentration, we believe that NMF was not formed from the photolysis of fluridone in our study ponds. Our findings suggest that NMF is not formed after the use of fluridone in aquatic systems and therefore the use of fluridone for aquatic plant control does not pose a health risk due to this compound. The results from our study agree with those obtained previously with radiolabeled fluridone (Berard and Rainey 1981).

Fluridone concentrations at the 0.5 m and 1.5 m stations within the ponds are illustrated in Figure 1. Fluridone concentrations were highest at the shallower stations immediately after treatment; this lasted until complete mixing took place after 35 days in Pond 1 and after 49 days in Pond 2. In turn, the dissipation of the fluridone was faster in shallow versus deep water. The half-life of the fluridone (time required for a 50% reduction in the initial concentration) was 15 and 17 days in Pond 1 (shallow and deep stations). In pond 2, a half-life of 16 and 19 days were determined for the shallow and the deep stations, respectively. These values were determined from a linear regression of fluridone concentration over time (Figure 1). Temperature and light intensity were reported as the major factors influencing the rate of fluridone dissipation (West *et al.* 1983). Within our ponds the half-life of fluridone approximated the medium of 21 days reported by West *et al.* (1983). The average residual concentration in Pond 1 was 15.3% of the initial concentration after 14 days and in Pond 2, after the second treatment, the average residual concentration was 13.2% of the initial after 35 days (Figure 1). The higher dissipation observed in Pond

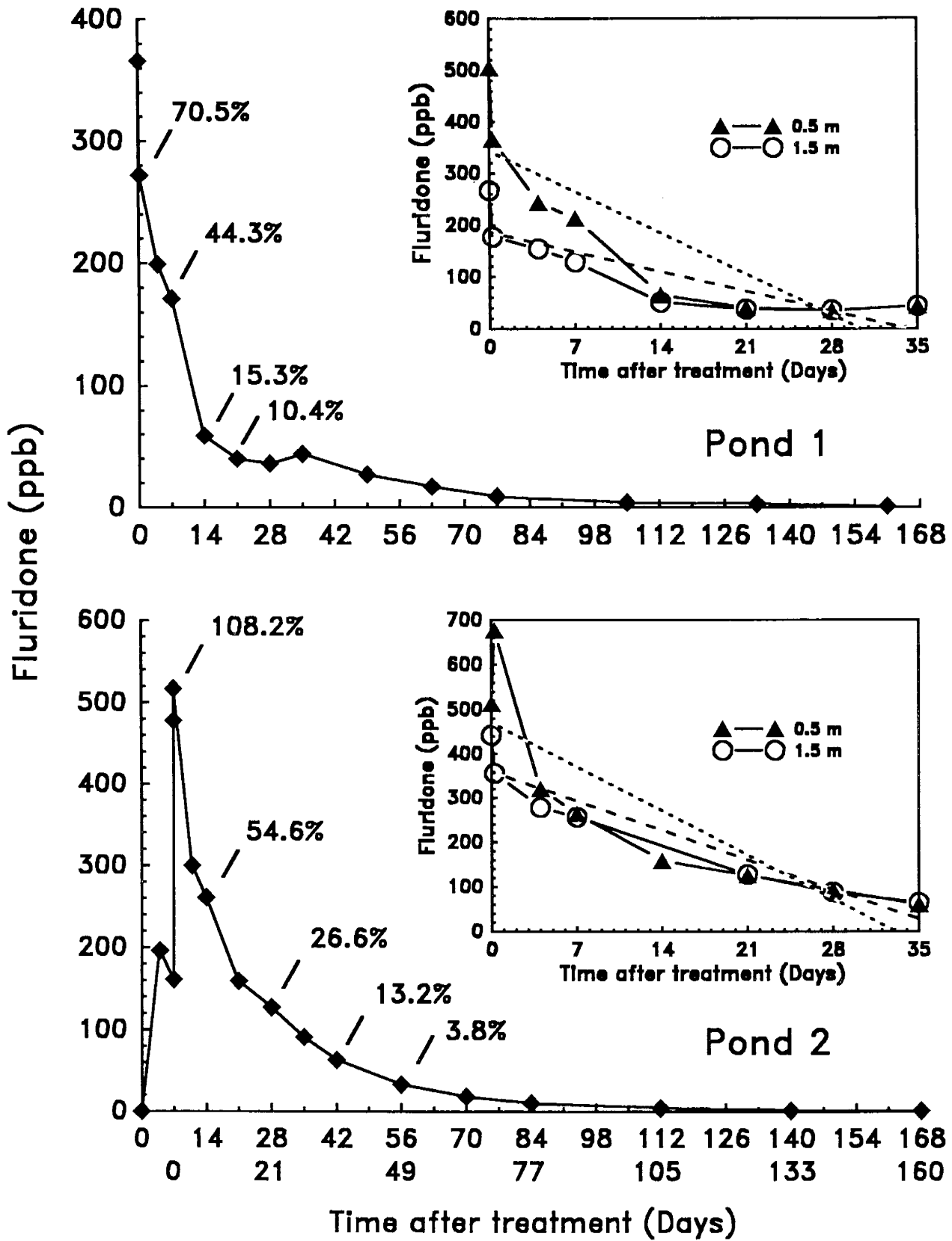


Figure 1. Dissipation of fluridone (ppb) in Experimental Ponds 1 and 2 at water depths of 0.5 and 1.5 m during the 35 day period after the application of fluridone; half life was determined from linear regression (broken lines) of fluridone concentration vs. time. These graphs are nested within illustrations of Pond 1 and 2 for the average fluridone concentration (ppb) and percent of initial fluridone concentration remaining per day throughout the 167 day study.

I could not be accounted for by differences in water quality (light, temperature, nutrients) between the ponds (Table 1). While the split treatment conducted in Pond 2 initially produced a sustained high concentration, the split treatment did not prolong the longevity of the herbicide since the time required for it to decline to a concentration of 1 ppb was nearly the same in both ponds (161 days, Pond 1; 167 days, Pond 2) Figure 1.

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# Allelopathic Potential of Sixteen Aquatic and Wetland Plants

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#### ABSTRACT

Aqueous extracts of 17 selected hydrophytes were tested for allelopathic activity using lettuce seedling and duckweed assay systems. Results from the lettuce seedling method were: all plant extracts showed statistically significant growth inhibition at the highest extract concentration tested; 15 extracts showed inhibition at the mid concentration tested; six extracts showed inhibition at the lowest concentration tested. Duckweed frond growth was inhibited by 12 of the 16 plant extracts at the highest extract concentration and by five of 16 at the mid concentration. Comparisons of the six most inhibitory plant extracts showed three of the six are common to both assays.

*Key words:* Duckweed, lettuce seedling assay, growth inhibition, hydrophytes, aqueous extracts, bioassays, chlorophyll.

#### INTRODUCTION

Allelopathy plays a role in determining the distribution and growth of higher plants (Rice, 1979). There are numerous reports of the allelopathic interactions of terrestrial plants, but much less is known about these relationships among hydrophytes. Dwarf spikerush plants have frequently been reported as being allelopathic (Yeo, 1980; Frank and Dechoretz, 1980; Yeo and Thurston, 1984; Ashton *et al.*, 1985; Nichols and Shaw, 1983) as has cattail (Szczepanski, 1977; Bonasera *et al.*, 1979; McNaughton, 1968; Szczepanska, 1971) (See Table 1 for listings of common and scientific names). Some publications report allelopathic activity based on field observations (dwarf spikerush and spikerush) (Nichols and Shaw, 1983) while others are based on plant extract activity (dwarf spikerush, water shield) (Frank and Dechoretz, 1980; Ashton *et al.*, 1985; Elakovich and Wooten, 1987) making it difficult to draw conclusions as to the most promising allelopathic hydrophytes. Szczepanska (1971) investigated the allelopathic interactions of roseacane, cattail, bullrush, and horsetail. She found plant production different in mixed cultures than in monospecific cultures, but the extent of influence

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