Distribution and Persistence of Diflubenzuron within Littoral Enclosure Mesocosms

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The insecticide diflubenzuron was applied as Dimilin 25W twice, 32 days apart, to the surfaces of 12 of 18 littoral enclosure mesocosms (5 × 10 m) to study its distribution, persistence, and mass balance in a natural ecosystem. Nominal concentrations were 0.7, 2.5, 7, and 30 μg/L active ingredient. The residue half-life in the water column ranged from 3.3 to 8.2 days with a mean of 4.3 days and required 14–35 days for 95% dissipation. The half-life in the macrophytes ranged from 2.0 to 5.7 days, and 95% dissipation required 8.6–24.6 days. The half-life in sediment ranged from 6.2 to 10.4 days, and 95% dissipation required 26.9–45.0 days. The water was the major compartment for residues, with amounts ranging from 82.3% of that applied after 3 h to 11.6% after 7 days. The sediment and macrophytes had maximum amounts of 6.3 and 10.2%, respectively. The mass balance ranged from 82.3% after 3 h to nondetectable after 56 days.

Keywords: *Diflubenzuron; persistence; mass balance; littoral enclosures*

INTRODUCTION

Diflubenzuron [1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea], the active ingredient of Dimilin 25W, is a chemical used to control numerous forest and agricultural pests and is most often applied by aerial spraying (Fischer and Hall, 1992; Retnakaran and Wright, 1987; Retnakaran et al., 1988). Diflubenzuron was selected for study because of its wide distribution, the potential for aquatic exposure from aerial spraying in forestry practices, the expansion of available data on the environmental persistence and distribution in freshwater ecosystems, and its unique mode of action. Diflubenzuron is a selective insect growth regulator which acts by inhibiting chitin formation and represents a class of pesticides that has never been tested using the littoral enclosure protocol (Brazner et al., 1989). The attributes of diflubenzuron include excellent target species control, low bioconcentration potential, and relative nonpersistence in the environment (Sundaram et al., 1991; Colwell and Schaefer, 1980; Madder and Lockhart, 1980; Schaefer et al., 1979; Booth and Ferrell, 1977). It is, however, toxic to select aquatic invertebrates (Fischer and Hall, 1992; Eisler, 1992; Sundaram et al., 1991; Apperson et al., 1978).

The environmental fate of diflubenzuron has been studied in the laboratory and field. The parent compound is moderately stable and the degradation and/or loss from the system is primarily dependent on pH, temperature, and the presence of organic matter and is less dependent on biodegradation and photolysis (Ivie et al., 1980; Schaefer and Dupras, 1976). The major degradation products of diflubenzuron are 2,6-difluorobenzamide, 2,6-difluorobenzoic acid, 4-chlorophenylurea, and 4-chloroaniline (Ivie et al., 1980; Schaefer et al., 1980; Metcalf et al., 1975). The most rapid degradation (half-life of 2.1 days) of diflubenzuron occurs at a combination of high pH (10) and high temperature (38 °C) (Schaefer and Dupras, 1976). These temperatures may be achieved in shallow stagnant pools typical of mosquito habitat; however, pH values ≥10 are not commonly found in natural waters (Hem, 1970). The degradation in field waters observed by Schaefer and Dupras (1976) at pH 7.7 and 24 °C was considerably slower with a half-life of 35 days. Adsorption onto the macrophytes, sediments, and wall material of the enclosures may be a more important dissipation pathway than hydrolysis. Past littoral enclosure studies with chlorpyrifos (Knuth and Heinis, 1992) and esfenvalerate (Heinis and Knuth, 1992) have shown the importance of sediment in sequestering hydrophobic chemicals for long periods of time (>1 year) and the importance of the macrophyte community in the mass balance, respectively. In a study conducted in a forest ecosystem, aerial spraying of diflubenzuron at a rate of 70 g of active ingredient (ai)/ha resulted in pond water concentrations of 13.8 and 5.90 μg/L (Sundaram et al., 1991). Residue levels of diflubenzuron in that study declined to ≤0.05 μg/L within 16–20 days in the water, 7–10 days in the aquatic vegetation, and 3 days in the sediment. Diflubenzuron persisted for 14 days in a study conducted in farm ponds treated with 2.5, 5, and 10 μg/L and for 35 days in a small lake that was treated with 5 μg/L; however, no diflubenzuron was detected in the sediment (Apperson et al., 1978). All of the water bodies had pHs ranging from 7.7 to 8.6 and temperatures from 20 to 25 °C, providing evidence that diflubenzuron does not degrade rapidly in natural waters of moderate pH and temperature.

Although several studies have assessed the aquatic persistence and effects of diflubenzuron, no comprehensive study describing the distribution, persistence, and mass balance in a freshwater ecosystem appears to exist. Recent literature reviews of fresh and estuarine waters by Fischer and Hall (1992) and Cunningham (1986) have concluded that limited data exist on diflubenzuron distribution in water, sediment, and leaf litter, thus limiting the potential to predict environmental effects due to diflubenzuron exposure. They also

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0021-8561/95/1443-1087$09.00/0

Table 1. Littoral Enclosure Treatment

treatment level	no. of replicate enclosures	application rate[a] (kg/ha)	mass applied[a] (mg)	nominal concn[b] (μg/L)
1	6	0.0	0.0	0.0
2	2	0.004	20.5	0.7
3	4	0.020	99.7	2.5
4	2	0.039	176	7
5	4	0.21	941	30

[a] The application rate and mass applied are reported as the mean of all replicate enclosures at each respective treatment level of both applications. [b] Nominal concn = (mass applied/volume of water).

concluded that many freshwater toxicity studies did not quantify the diflubenzuron after application. This study was specifically designed to address these inadequacies, to obtain a more complete understanding of the environmental dissipation, distribution, and mass balance of diflubenzuron in a littoral ecosystem, and to provide exposure data for concurrent biological effects studies, fate and effects modeling efforts, and risk assessment evaluation. This study is one in a series of field studies (Heinis and Knuth, 1992; Knuth and Heinis, 1992; U.S. Environmental Protection Agency, 1992) that utilize littoral enclosures (Brazner et al., 1989) as a model ecosystem for comprehensive, cost effective, integrated, chemical, biological, and ecological effects studies. The littoral enclosure protocol incorporates replication into the experimental design, improving the statistical analysis and interpretation of results.

MATERIALS AND METHODS

Study Site. Eighteen enclosures (5 × 10 m) were previously constructed in a 2-ha pond near Duluth, MN (46° 52′ 00″ N, 92° 10′ 00″ W) (Brazner et al., 1989). Each enclosure included 5 m of natural shoreline and three walls constructed of an inert polyolefin plastic (888 HUV Clear Scrimweve, Sto-Cote Products Inc., Richmond, IL). The littoral areas were well developed with cattails, pond grasses, and aquatic macrophytes growing in highly organic sediments. *Chara* species dominated the submerged aquatic vegetation. The enclosures (n = 18) had an average surface area (±SD) of 46.3 ± 9.7 m^2 (range 31.9–55.6 m^2), an average depth of 0.7 ± 0.2 m (0.5–1.1 m), and an average water volume of 32.8 ± 6.3 m^3 (16.5–54.2 m^3).

Experimental Design. The enclosures were constructed in three blocks of six enclosures each. The overall experimental design consisted of two blocks with two control enclosures and one enclosure at each of the following treatment concentrations: 0.7, 2.5, 7, and 30 μg of active ingredient/L. These nominal concentrations were selected on the basis of their anticipated biological and ecological effects. The remaining block had two control enclosures, two enclosures at 2.5 μg/L, and two enclosures at 30 μg/L diflubenzuron. Water column residues were measured in all enclosures. The distribution, persistence, and mass balance of diflubenzuron was measured in one enclosure treated at 2.5 μg/L and in another treated at 30 μg/L. The two treatment levels were selected to provide distribution, persistence, and mass balance information across the range of concentrations at which biotic effects would likely occur.

Pesticide Application. Two applications of diflubenzuron as the wettable powder formulation Dimilin 25W (25% active ingredient by weight) were made to the littoral enclosures (Table 1). Applications occurred on July 9 and August 11, 1992, respectively, between 9:00 a.m. and 1:30 p.m. (CDT). The pesticide was applied to the surface of the littoral enclosures using a portable hand sprayer with a 2.4 m wand. The mass of Dimilin wettable powder required to achieve the nominal concentrations in each enclosure was weighed in the laboratory prior to application. At the study site, the Dimilin wettable powder was mixed with 4 L of distilled water in the sprayer tank and pressurized. The tank mixture was applied evenly over the entire surface within the enclosure by making several uniform passes with the sprayer wand. Application was performed in increasing order of concentration and took approximately 15 min for each enclosure. Care was taken during the application procedure to avoid spray drift and uneven surface distribution.

Sample Collection. Depth integrated composite water samples were collected from all enclosures biweekly from June 17 to September 7, 1992, for pH, conductivity, turbidity, apparent color, alkalinity, dissolved organic carbon (DOC), and total organic carbon (TOC) analyses. DOC samples were filtered through a prewashed (50 mL of deionized water, 50 mL of sample) 0.45 μm Millipore HA membrane filter prior to analysis. Depth integrated composite water samples for diflubenzuron analysis were collected from one control and each treatment enclosure at 1, 3, and 9 h and at 1, 2, 4, 7, 14, and 32 days after each pesticide application. The composite samples consisted of five subsamples: four taken at each corner of the open water area approximately 0.5–1 m from the side walls and one taken from the center of the enclosure.

The homogeneity of diflubenzuron concentration following application was investigated by taking top and bottom water samples simultaneously at 1, 3, 9, and 24 h from approximately 5 cm below the water surface and 5 cm above the sediment surface, respectively, of enclosures 2 and 7 (30 μg/L). Samples were taken at a single location at the deepest end of the enclosure.

In situ sediment sample containers were used to collect sediment for study as described by Knuth and Heinis (1992). The sediment containers were placed on the pond bottom within two enclosures 19 days before the first diflubenzuron application. Subsamples of the bulk composite sediment were used as preapplication samples and for grain size analysis. After deployment at the field site, duplicate containers of sediment were collected at 3 and 9 h and 1, 2, 4, 7, 14, and 32 days after application 1 and at 3 and 9 h and 1, 2, 4, 7, 14, 32, and 56 days after application 2. This allowed for a measure of precision for each sampling date and each sample type and encompassed the entire range of diflubenzuron residue concentrations encountered in the study.

Submerged macrophytes (predominantly *Chara* spp.) were collected using a grab technique (Vollenweider, 1974). A 10 cm × 10 cm rake with a 1.4 m handle was dropped through the algal mat until the sediment bed was reached. The rake was turned 90°, raised slowly 7–10 cm, and jerked to dislodge the samples from the macrophyte bed. The area sampled was approximately 360 cm^2 per grab sample. One shallow grab (< mean depth) and one deep grab (> mean depth) were collected from each enclosure. The two grab samples were drained of excess water, combined, placed into a wide-mouth glass jar, stored on ice, and transported to the laboratory. A subsample was removed for moisture determination. The wet weight of the remainder of the sample was determined, and the sample was stored (−20 °C) until extraction and analysis.

Sample Extraction. Water samples for the determination of diflubenzuron concentrations were extracted using solid-phase extraction techniques. Octadecyl (500 mg C_{18}, 3 mL) columns were prepared by successive washes of 5 mL of acetonitrile, 5 mL of methanol, and 5 mL of deionized water. Sample volumes ranged from 100 to 500 mL depending on the expected concentration. Samples with excessive particulates or sample volumes of 400 mL or more were prefiltered using 27 mm glass fiber filters prior to the C_{18} column extraction. Prefiltering had no effect on diflubenzuron recovery. The filter apparatus was removed (if incorporated), following sample addition, and the columns were rinsed with 35 mL of 30% acetonitrile in deionized water (v/v). Diflubenzuron was eluted from the extraction column with 2 mL of acetonitrile and collected in a graduated centrifuge tube. The sample was evaporated to dryness in a hot water bath using a stream of dry nitrogen gas. The residue was redissolved by adding 0.5 mL of methanol followed by 0.5 mL of deionized water with vortex mixing after each addition.

The methods utilized in the extraction of sediment and macrophytes were modified from those of DiPrima et al. (1978). The frozen sediment sample was thawed overnight. An aliquot (1–2 g of wet weight) of sediment was retained for moisture determination and TOC analysis (U.S. Environmental Protection Agency, 1987). The top 1 cm (≈50 g of wet weight) of sediment was removed from the sample container and was transferred to a 6 cm glass filling funnel atop a 500 mL boiling flask. The sediment was fortified with 0.5–1.0 mL of a methanol solution containing the surrogate, linuron [3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea, 985 ng/mL], and allowed to air-dry for 10 min. The sample was transferred to the boiling flask with 200 mL of 85% acetonitrile/distilled deionized water (DIW) (v/v). The sample was refluxed for 30 min, cooled until it could be handled safely, and filtered through Whatman Grade 1 filter paper in a fluted glass funnel into a 250 mL beaker. Filtrate (100 mL) was transferred into a 250 mL Kuderna-Danish evaporative concentrator (K-D) and concentrated to a volume of 8 mL. The concentrated filtrate was transferred to a 500 mL separatory funnel with 85 mL of DIW and partitioned with hexane (3 × 50 mL). The hexane layers were combined in a 250 mL K-D through a 6 cm o.d. fluted funnel packed with a glass wool plug and 25 g of anhydrous Na_2SO_4. The hexane extract was concentrated to 1 mL by K-D followed by nitrogen blowdown using an N-EVAP evaporator concentrator (Organomation Associates Inc., Berlin, MA).

Interfering substances in the sediment extract were removed by liquid–solid chromatography. A 1 cm × 30 cm glass column with a 300 mL reservoir was packed with a glass wool plug and 2 cm of anhydrous Na_2SO_4, which was covered with 10 mL of hexane. The sorbent, 12 g of florisil (activated at 105 °C), was combined with 50 mL of hexane in a 200 mL beaker, stirred, and allowed to stand for 1 min. The slurry was stirred and poured into the chromatography column through a glass filling funnel. Any sorbent remaining in the beaker or funnel was rinsed onto the column with packing solvent. Anhydrous granular Na_2SO_4 (3 cm) was added to the top of the column, and the walls of the column were rinsed with packing solvent. The solvent in the column was drained to the top of the Na_2SO_4, and then the sample extract was applied with 3 × 2 mL dichloromethane rinses. The column was rinsed with 100 mL of hexane, 30 mL of 10% acetone/hexane (v/v), and 10 mL of 20% acetone/hexane (v/v). The rinse solutions were discarded, and the column was eluted with 100 mL of 20% acetone/hexane (v/v) into a 250 mL K-D. The eluent was concentrated to dryness, redissolved in 2 mL of methanol, and mixed for 1 min on a Genie vortex mixer (American Hospital Supply Corp., Scientific Products Division, Evanston, IL).

The frozen macrophyte samples were thawed in their storage containers, and any excess free water was allowed to drain into a stainless steel pan. Each wet plant sample (10 g maximum weight) was transferred to a 1 L glass blender jar, fortified with 0.5–1.0 mL of a methanol solution of the surrogate (linuron), and allowed to air-dry for 10 min, and then 150 mL of 15% DIW/acetonitrile (v/v) was added. The mixture was blended for 10 min at low speed. The blended sample was filtered through Whatman Grade 1 filter paper in a fluted glass funnel into a 250 mL beaker. The blender was rinsed with 50 mL of 15% DIW/acetonitrile (v/v) into the filter. The remainder of the extraction procedure was identical to the sediment extraction procedure detailed above.

Interfering substances in the macrophyte extracts were removed by liquid–solid chromatography, as above, using 25 g of 10% deactivated (v/w) Florisil as sorbent, a 150 mL hexane rinse, and 200 mL of 10% acetone/hexane (v/v) as eluent. The eluent was concentrated to dryness and redissolved in 2 mL of methanol as above.

Sample Analysis. Alkalinity titrations were performed using a Radiometer DTS 800 series digital titration system, and concentrations were calculated by Gran function regressions (Seymour, 1978). DOC and TOC were analyzed using a Dohrman DC-90 automated carbon analyzer utilizing ultraviolet-promoted persulfate oxidation. Samples were acidified to pH <2 with H_2SO_4 and sparged with oxygen in the autosampler to remove inorganic carbon. Diflubenzuron was analyzed on a Hewlett-Packard model 1084B HPLC equipped with a Brownlee ODS Spheri-5 C_{18}, 30 mm × 4.6 mm, 5 μm precolumn and a Hewlett-Packard Hypersil ODS C_{18}, 100 mm × 4.6 mm, 5 μm analytical column. A variable-wavelength UV detector was operated at 278 nm and a standard injection volume of 100 μL. Water extracts were analyzed isocratically at a flow rate of 2.0 mL/min. The mobile phase was 70% methanol/30% deionized water (v/v). Sediment extracts were analyzed using a flow rate of 1 mL/min throughout the run and a solvent gradient elution as follows: 65% methanol/DIW (v/v) initially, increased to 75% methanol by 6 min, held at 75% methanol until 7 min, increased to 85% methanol at 9 min, held at 85% methanol until 11 min, reduced to 65% methanol at 13 min, and held at 65% methanol through the end of the run at 14 min. Macrophyte extracts were analyzed using a flow rate of 0.75 mL/min throughout the run and a solvent gradient elution as follows: 70% methanol/DIW (v/v) initially, held for 8 min, increased to 85% methanol by 10 min, held at 85% methanol through 12 min, reduced to 70% methanol at 14 min, and held at 70% methanol through the end of the run at 15 min.

Quantitation and Quality Assurance. The extraction efficiency of diflubenzuron from enclosure water was evaluated by fortifying untreated enclosure water with diflubenzuron at the field site. Reported water diflubenzuron concentrations were not corrected for extraction efficiency. Method precision was assessed by analyzing duplicate samples, taken from the same enclosure at the same time, at each sampling event.

The extraction efficiency of diflubenzuron from the sediment and macrophytes was evaluated by adding known amounts of a surrogate chemical, linuron, to each sample matrix before extraction. Prior to the study, blank sample matrices were fortified with diflubenzuron and linuron to verify comparable extraction efficiency of the analyte and surrogate. Peaks were identified by retention time and verified biweekly by fortifying standards and extracts with known amounts of diflubenzuron and linuron. Peak heights were measured and sample concentrations were determined by linear regression using four external standards containing diflubenzuron for water extracts and diflubenzuron and linuron for sediment and macrophyte extracts. Standard solutions were prepared in 50% methanol/deionized water (DIW).

Reported sediment and macrophyte diflubenzuron concentrations were corrected for extraction efficiency based on the surrogate recovery. Duplicate, blank, and fortified samples were analyzed at a frequency of no less than 8% to assess the precision and accuracy of the method with respect to each sample matrix and to determine detection and quantitation limits of diflubenzuron (Table 2). The quantities of diflubenzuron measured in the sediment and macrophytes of the littoral enclosures were normalized on a dry weight basis for comparisons of concentration gradients and to detect trends in dissipation and persistence of diflubenzuron.

RESULTS AND DISCUSSION

Physical and Chemical Measurements. Analyses of physical and chemical parameters for enclosures were performed to characterize the site water (Table 3). The sediment was 79.2% sand with particle size >50 μm, 18.3% silt with particle size from 2.0 to 50 μm, and 2.5% clay with particle size from 0.08 to 2.00 μm. The total organic carbon content of the sediment samples ranged from 3.6 to 7.6% with a mean and standard deviation of 5.8 and 1.0%, respectively ($n = 16$).

Precision and Accuracy of Application. Precision, defined here as how reproducible the measured concentrations of diflubenzuron were at each respective treatment level, was assessed by calculating the coefficient of variation (CV). The CV following application 1 ranged from 12.6% in the 7 μg/L treatment to 32.9% in the 2.5 μg/L treatment, with a mean CV for all

Table 2. Measures of Dispersion Pertinent to Diflubenzuron Residue Analysis

type	water (μg/L)	sediment (μg/kg)	macrophytes (μg/kg)
LLD[a]	2.00 ng	2.00 ng	2.00 ng
MDL[b]	0.12	1.07	2.94
95% CL[c]	0.08–0.26	0.69–2.35	1.88–6.46
n	13	7	7
fortified samples	diflubenzuron % recovery	surrogate % recovery	surrogate % recovery
mean	100	87.2	112
SD	20.6	17.0	15.0
% CV	20.6	19.5	13.4
min	69.4	62.8	82.6
max	176	130	143
n	29	47	17
duplicate samples[d]		RPD[e]	
mean	15.3	40.0	32.9
SD	12.4	29.8	32.1
% CV	81.0	74.6	97.3
min	2.90	0.57	6.29
max	50.0	100	86.6
n	13	15	5

[a] Lower limit of instrument detection, based on 3 mm peak height, 100 μL injection volume. [b] Method detection limit determined by the method of Glaser et al. (1981). [c] 95% confidence interval (Glaser et al., 1981). [d] Two samples collected from the same enclosure at the same sampling time. [e] Relative percent difference [((high value − low value)/high value) × 100].

Table 3. Water Quality Data of Littoral Enclosures

parameter[a]	units	mean	SD	min	max	n
conductivity	μs/cm	238	75.3	118	510	93
pH		8.1		7.3	9.1	92
alkalinity	mg/L $CaCO_3$	134	61.7	57	314	90
color	PCU	32	10.4	20	65	76
turbidity	NTU	1.1	0.7	0.1	3.9	88
DOC	mg/L C	12	4.1	7	28	107
TOC	mg/L C	12	4.0	7	28	108
temperature	°C	19.5	2.35	14.1	23.3	85

[a] Measurement period was June 17–Sept 7, 1992.

treatments over the entire time diflubenzuron was detected of 21.8%. Application 2 had similar variability, and the CV ranged from 8.4% in the 7 μg/L treatment to 35.6% in the 0.7 μg/L treatment with a mean of 24.5%. The 7 μg/L treatment had the best precision of all treatment levels. The overall study CV at all sample times and treatment levels following both applications was 23.2% ($n = 41$).

The variability of diflubenzuron concentration within replicate treatment enclosures was similar to previous pesticide studies. The overall CV for the organophosphorus pesticide chlorpyrifos was 17.7% ($n = 32$) (Knuth and Heinis, 1992). Two successive applications of the synthetic pyrethroid esfenvalerate had CV values of 31.7 ($n = 11$) and 39.4% ($n = 15$), respectively. The organophosphorus pesticide azinphos-methyl was studied for two consecutive years with a single application each year. The overall CVs for those studies were 24.0 ($n = 16$) and 28.5% ($n = 8$), respectively (U.S. Environmental Protection Agency, 1992).

The accuracy of the pesticide concentrations, defined here as the agreement between the measured concentrations and the nominal values, is an important element of field mesocosm studies to ensure a valid test and enhance interpretation of biological data. The accuracy was assessed by calculating the mean measured concentration of all replicate enclosures at 1 h after each treatment and calculating the relative percent difference [(nominal − actual/highest) × 100] with the nominal values. The relative percent difference ranged from 29% above nominal in the 0.7 μg/L treatment to 39% below nominal in the 30 μg/L treatment following application 1. The mean deviation was 10.5% ($n = 4$) below nominal. The range after application 2 was 14% below nominal in the 0.7 μg/L treatment to 43% below nominal in the 7 μg/L treatment. The mean deviation was 32% ($n = 4$) below nominal. The overall deviation considering all treatment levels and both applications was 21% ($n = 8$) below nominal. There was only one treatment (0.7 μg/L, application 1) that was above nominal, and there was a general tendency that the measured and nominal concentrations agreed better in the lower treatments.

Residues within the Water Column. Theoretically, the maximum concentration of diflubenzuron or other surface-applied water miscible toxicant should occur at the time of application. To assess this and when the first measurable declines in pesticide occurred, additional water samples were taken at 3 and 9 h from enclosures 11 (2.5 μg/L), 2, and 7 (30 μg/L) following application 1 and from enclosures 11 and 7 following application 2. The concentrations dropped on average 15% for these enclosures during the first 24 h after treatment. At 3 and 9 h deviations from the 1 h concentrations were 7 and −5%, respectively. These data indicate that diflubenzuron has a fairly constant concentration in the water during the first 9 h. Concentrations dropped an average of 34% between 1 and 24 h considering all treated enclosures. Diflubenzuron concentrations were measured similarly following application 2, where they dropped an average of 37% between 1 and 24 h. The extended initial concentration observed is not unusual. Previous mesocosm studies with esfenvalerate (Knuth and Heinis, 1992) and azinphos-methyl (U.S. Environmental Protection Agency, 1992) have shown similar initially constant concentrations at various treatment levels. Sundaram et al. (1991) studied diflubenzuron (WP-25) in forest ponds and streams following aerial application and in one of two ponds observed a constant concentration for 6 h, which then declined 53% by 24 h. The measured concentrations immediately following application may be influenced by pesticide formulation, climatic conditions (i.e. surface wind velocity), uneven application, incomplete mixing, sampling bias, and diffusion and adsorption processes (Sundaram et al., 1991; Cunningham, 1986; Mian and Mulla, 1982). The influence of these is specific to the toxicant and the site or study conditions.

Diflubenzuron was undetectable (MDL = 0.12 μg/L) at 7 days following application 1 in the lowest treatment (0.7 μg/L) and between 14 and 32 days in the highest treatment (30 μg/L). Dissipation was similar after application 2, with measured values in the highest treatment nearly equal to the detection limit at 32 days (Figure 1). This dissipation pattern agrees well with a similar pond experiment by Colwell and Schaefer (1980) in which measured diflubenzuron residues of 13.2 μg/L at 1 h posttreatment were below detectable limits (<0.2 μg/L) by 14 days. Sundaram et al. (1991) found that 1 h diflubenzuron residues of 5.9 and 13.8 μg/L persisted for 15 days in freshwater ponds aerial sprayed at 70 g/ha (MDL = 0.05 μg/L). These studies indicate that diflubenzuron residues in water of approximately 0.7–30 μg/L will be detectable for about 7–32 days after application.

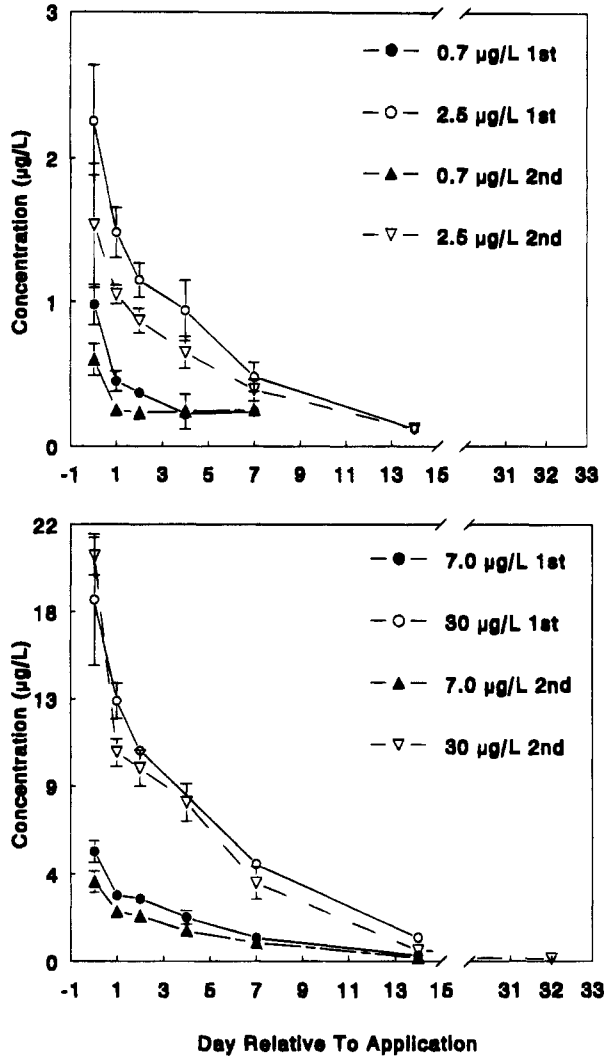

Figure 1. Dissipation of diflubenzuron from littoral enclosure water at four treatment levels following applications 1 and 2. Each point represents the mean of all enclosure concentrations (n = 2 for 0.7 and 7 µg/L; n = 4 for 2.5 and 30 µg/L). Each error bar represents one standard deviation about the mean.

The loss rate and persistence of diflubenzuron in the treated enclosure water was determined by calculating the half-life (DT_{50}) and the time to 95% loss (DT_{95}). The loss of diflubenzuron at treatment levels of 2.5 µg/L and above fits first-order kinetics and was accurately described by linear regression analysis of ln concentration (c) vs time (t) (Table 4). Coefficients of correlation (r^2) in these treatments were all greater than 0.9. The correlation coefficient of the 0.7 µg/L treatment levels was the lowest, with r^2 values of 0.68 and 0.32 for applications 1 and 2, respectively. The low correlation in the 0.7 µg/L treatments was due to an initial concentration drop of 58 to 54%, after applications 1 and 2, respectively, by 24 h. The concentration then remained nearly constant at twice the detection limit until residues were undetectable at 7 days. The linear regression estimates of the DT_{50} (8.2) and DT_{95} (35.6) after application 2 are probably not accurate. However, the DT_{50} and DT_{95} values for the 0.7 µg/L treatment following application 1 were in excellent agreement with those of higher treatment levels (Table 4).

The DT_{50} and DT_{95} values between applications 1 and 2 agreed well at the 2.5, 7, and 30 µg/L treatment levels (Table 4). The second application values were higher due to the poor correlation at the 0.7 µg/L treatment.

Table 4. Estimated DT_{50} and DT_{95}[a] Values for Diflubenzuron in Littoral Enclosure Water following Applications 1 and 2

treatment level (µg/L)	equation[b]	r^2	DT_{50} (days)	DT_{95} (days)
	Application 1			
0.7	$\ln c = -0.447 - 0.179t$	0.685	3.9	16.7
2.5	$\ln c = 0.602 - 0.204t$	0.962	3.4	14.7
7	$\ln c = 1.57 - 0.203t$	0.991	3.4	14.8
30	$\ln c = 2.82 - 0.187t$	0.995	3.7	16.0
		mean =	3.6	15.6
	Application 2			
0.7	$\ln c = -1.01 - 0.0842t$	0.321	8.2	35.6
2.5	$\ln c = 0.286 - 0.174t$	0.992	4.0	17.2
7	$\ln c = 1.28 - 0.211t$	0.991	3.3	14.2
30	$\ln c = 2.55 - 0.157t$	0.932	4.4	19.1
		mean =	5.0	21.5

[a] Time (days) for 50% and 95% dissipation, respectively. [b] From a least-squares regression of ln concentration (c) vs time (t). Equations were derived using the mean concentration of all replicate enclosures at each treatment level (0.7 and 7 µg/L, n = 2; 2.5 and 30 µg/L, n = 4).

Excluding this treatment level from application 2 results in mean values of 3.9 ± 0.6 (n = 3) and 16.8 ± 2.5 days (n = 3), respectively. The DT_{50} and DT_{95} values considering all treatment levels of both applications ranged from 3.3 to 8.2 and from 14.2 to 35.6 days, respectively, and the mean values were 4.3 ± 1.6 (n = 8) and 18.5 ± 7.1 days (n = 8), respectively.

The persistence of diflubenzuron has been measured by other researchers. Sundaram et al. (1991) investigated the persistence of diflubenzuron (WP-25) in freshwater ponds with depths similar to those of the littoral enclosures used in this study. The diflubenzuron loss from the water column in two ponds was rapid, with DT_{50} values of 0.4 and 1.3 days, respectively. The DT_{95} values were 1.4 and 4.2 days, respectively. The 1 h measured concentrations of diflubenzuron in these ponds were 13.8 and 5.9 µg/L, respectively, which is nearly equal to the highest two treatments of the enclosure study. The persistence, however, is less than that found in the littoral enclosures. Probable causes for the observed difference may be the water sampling location and higher turbidity. Sundaram et al. (1991) took samples from the top 5 cm of water; thus, initial mixing of the applied diflubenzuron would cause rapid loss in the top layer of water, contributing to the apparent short persistence. Turbidities in the ponds studied by Sundaram et al. (1991) were 12 and 60 JTU compared to 1.1 NTU (a comparable unit) in the littoral enclosures. This greater turbidity could enhance diflubenzuron adsorption and subsequent loss from the water column.

The stability and degradation of diflubenzuron are well described by Schaefer and Dupras (1976) and Ivie et al. (1980). Hydrolysis and adsorption to organic matter were the major processes affecting diflubenzuron persistence. Photolytic and microbial degradation are of minor importance (Schaefer and Dupras, 1976). Diflubenzuron persistence was dependent on pH and temperature, exhibiting no degradation at pH 4 to a half-life of <3 days at pH 10 (Ivie et al., 1980). The combined effect of high temperature and high pH provides the most favorable conditions for diflubenzuron degradation. The persistence of diflubenzuron observed in this study was in agreement with the observations of Ivie et al. (1980) and Schaefer and Dupras (1976).

Table 5. Concentration at the Top and Bottom of the 30 μg/L Enclosure Water Column

enclosure no.	depth[a]	time after application 1 h	3 h	9 h	24 h
2	top	170.0	59.8	49.7	12.7
	bottom	3.09	17.0	12.8	8.5
7	top	82.8	58.7	13.4	11.6
	bottom	4.96	1.30	0.93	11.5

[a] Sampled approximately 5 cm from water surface (top) and 5 cm from sediment surface (bottom). The maximum depths of enclosures 2 and 7 were 1.42 and 0.94 m, respectively.

The littoral enclosures had a mean pH of 8.15, and the mean water temperature during the time diflubenzuron was present was 21.1 °C. These pH and temperature conditions are not favorable for rapid degradation and, combined with the low particulate matter of the study water (1.1 NTU), would manifest the moderate diflubenzuron persistence we observed.

The concentration of surface-applied toxicant with respect to enclosure water depth has historically been nonhomogeneous for the first 24 h (Knuth and Heinis, 1992; Heinis and Knuth, 1992). Diflubenzuron acted similarly in that most of the residue was near the surface in the first hours following treatment. The surface and bottom water of enclosures 2 and 7 (30 μg/L treatments) were sampled at 1, 3, 9, and 24 h to document the extent of vertical distribution versus time following application (Table 5). At 1–9 h, 74–98% of the applied diflubenzuron was found in the top water samples. At 24 h, enclosure 7 was completely mixed, resulting in a final concentration of 11.6 μg/L. This concentration is nearly identical to the corresponding composite sample (11.9 μg/L) from this enclosure, confirming the homogeneous condition. The top contained 33% more diflubenzuron than the bottom at 24 h in enclosure 2, with a mean concentration of 10.6 μg/L which also agrees closely with the corresponding composite (11.5 μg/L). A majority of the mixing of diflubenzuron took place between 9 and 24 h posttreatment. In contrast, Schaefer and Dupras (1976) observed complete mixing within 1 h after application of WP-25 formulation; however, the study ponds were 27 cm deep, which would make vertical mixing effects difficult to discern. Apperson et al. (1978) documented the lack of vertical mixing in larger study ponds that were 3–5 m deep and treated with the WP-25 formulation. Maximum bottom water diflubenzuron concentrations were measured from 4 h to 14 days after application. As these ponds and our enclosures gradually mix, diflubenzuron residues constantly decrease, thus exposing bottom-dwelling organisms to lower concentrations than those in the surface water.

Residues within the Sediment. *Low Concentration (2.5 μg/L).* Trace quantities of diflubenzuron were measurable in the sediment of the 2.5 μg/L treatment concentration enclosure. After application 1 from days 1 to 7, amounts ranged between 0.45 and 0.78 μg/kg. After application 2, diflubenzuron was detectable from 9 h through 7 days with amounts of 0.59 and 0.27 μg/kg, respectively (Figure 2). The highest values occurred 4 days after application 1 and 9 h and 4 days after application 2. After each application, sediment diflubenzuron residue concentrations were below detection in the 14 day samples.

High Concentration (30 μg/L). The accumulation and dissipation of diflubenzuron in the sediment after application 1 were characterized by trace quantities of 0.85 and 0.61 μg/kg 3 and 9 h after application, respectively. A maximum of 16.6 μg/kg was present 4 days after application 1. By 32 days after application, the concentration had decreased to 0.59 μg/kg (Figure 2).

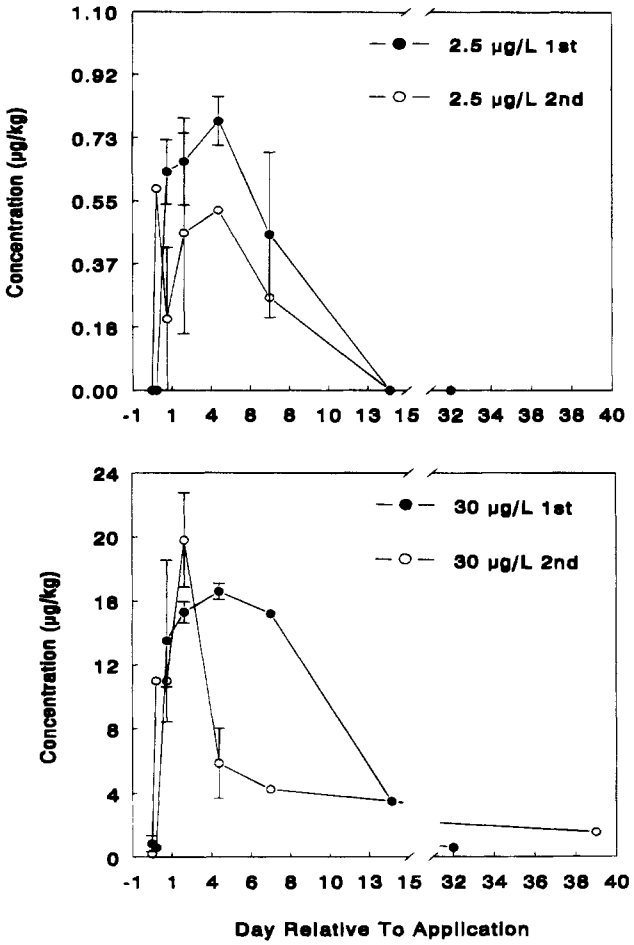

Figure 2. Diflubenzuron residue concentrations in the sediment of 2.5 and 30 μg/L littoral enclosures. Data where error bars are present are means of two duplicate samples collected at the same time from the same enclosure. Error bars indicate one standard deviation about the mean (n = 2).

Table 6. Estimated DT_{50} and DT_{95}[a] Values for Diflubenzuron in Littoral Enclosure Sediment

30 μg/L treatment level	equation[b]	r^2	DT_{50} (days)	DT_{95} (days)
application 1	$\ln c = 3.04 - 0.111t$	0.973	6.2	26.9
application 2	$\ln c = 2.39 - 0.067t$	0.721	10.4	45.0

[a] Time (days) for 50% and 95% dissipation, respectively. [b] From linear regression of ln concentration (μg/kg) vs time (days) (application 1, n = 4; application 2, n = 5).

Slightly increased amounts of diflubenzuron were measured in the sediment after application 2. By 9 h, 11.0 μg/kg was present, and a maximum amount of 19.8 μg/kg was measured 2 days after application. The amount decreased to 1.56 μg/kg after 32 days with nondetectable amounts 56 days after application 2.

The time to 50% (half-life) and 95% dissipation (DT_{50} and DT_{95}, respectively) was estimated using the linear regression of ln (diflubenzuron concentration in the sediment) (μg/kg) vs time (days) (Table 6). Values were independently derived for each application of diflubenzuron. Initial concentrations were based on the maximum values after each application. DT_{50} values were 6.2 and 10.4 days after applications 1 and 2, respec-

tively. DT_{95} values were 26.9 and 45.0 days after applications 1 and 2, respectively.

Longer persistence of a chemical in the sediment was also observed after the second of two applications of esfenvalerate in littoral enclosures (Heinis and Knuth, 1992). The longer persistence of diflubenzuron in the sediment after the second application was probably due to several factors. One factor was a slight residual diflubenzuron concentration remaining after the first application. The second was a slower observed dissipation rate in the water column after the second application (Table 4). DT_{50} values in the water were 3.7 and 4.4 days after applications 1 and 2, respectively. Another factor could have been a combination of environmental effects due to the date of the second application. The mean daily water temperature was 22.5 °C on July 9 and 20.9 °C on August 11, the dates of the first and second applications, respectively. This lower water temperature could have reduced the hydrolysis rate of diflubenzuron, leading to the observed longer persistence after the second application. Another factor that could decrease the degradation rate in the water column and therefore in the sediment could be a reduction in photolysis due to the lower angle of incidence of solar radiation and reduced duration of daylight after the second application with respect to the first.

Diflubenzuron persistence in sediment has varied among reported field studies, perhaps indicating that environmental conditions and method of application play important roles in the persistence. Sampling methodology and frequency may also play an important role in the estimation of persistence. Sundaram et al. (1991) studied diflubenzuron persistence and effects in forest ponds after aerial application and found measurable levels for only 3 days in one pond and for 2 days in another; consequently, no DT_{50} or DT_{95} values were calculated from those data. Apperson et al. (1978) found no measurable diflubenzuron residue in the sediment of a small lake receiving 5 μg/L as a surface application. Booth and Ferrell (1977) found no detectable levels in sediments from a small lake even after four diflubenzuron surface spray applications at 40 g/ha (~7 μg/L). Cunningham and Myers (1986), however, found that sediment was a major site for diflubenzuron adsorption in a supratidal saltmarsh, where persistence of diflubenzuron in the sediment generally increased after successive applications by fixed wing aircraft at a rate of 45 g/ha. Persistence in the sediment was generally >14 days and the half-life was estimated at 10 days.

The disparity of results can be explained primarily by the variability in the method detection limit and, in one case, by a combination of the sampling method and the test system. Sundaram et al. (1991) measured up to 240 μg/kg in the sediment of a forest pond but could only measure the chemical for 2 or 3 days before reaching their method detection limit of 50 μg/kg. Apperson et al. (1978), on the other hand, reported a method detection limit of 3.8 μg/kg but were unable to detect diflubenzuron in any of the sediment samples collected. However, in this case, the nominal treatment level was only 5 μg/L, the test system was 5 m deep, and the bottom waters did not contain any diflubenzuron until 3–4 days after application, when residue levels reached 1.3 to 3.3 μg/L there. The use of an Ekman grab sampler to collect the sediment may also have diluted the top portion of the sediment, which may have contained measurable amounts of diflubenzuron, with uncontaminated underlying sediment. Even after four applications of diflubenzuron at 40 g/ha, Booth and Ferrell (1977) reported the sediment residue below 50 μg/kg. They did not specify the depth of the test system, the application method, or the sampling method. Cunningham and Myers (1986) reported a sediment method detection limit of 10 μg/kg. After three aerial applications each of a 0.4% sand granule formulation and a 25% wettable powder formulation, sediment residues ranged from 10 to 100 μg/kg. Samples were collected as 6 cm diameter × 5 cm deep cores. No sediment DT_{50} or DT_{95} values were estimated by the authors. Stable water concentrations throughout the above study, coupled with a relatively shallow test system (1–2 m), contributed to the greater amounts of diflubenzuron measured in the sediment.

In the littoral enclosure study presented here, the method detection limit of 1.07 μg/kg allowed enough samples to be measured over time to provide an estimate of the DT_{50} and DT_{95} values, even though the maximum sediment concentration was only 19.8 μg/kg. The shallow test system (mean depth 0.7 m) and the container device sampler, combined with fractionation of only the top 1 cm of sediment for analysis, contributed to the low method detection limit achieved in this study. Interestingly, the DT_{50} values (5.6 and 10.7 days) obtained in this study were very similar to those obtained in previous littoral enclosure studies with esfenvalerate (Heinis and Knuth, 1992), chlorpyrifos (Knuth and Heinis, 1992), and azinphos-methyl (U.S. Environmental Protection Agency, 1992), for which DT_{50} values were 6, 10, and 6 days, respectively. Since the above chemicals have log K_{ow} values of 6.77, 4.82, and 2.62, respectively, and diflubenzuron has a log K_{ow} of 3.54 (Metcalf et al., 1975), there appears to be little dependence of half-life on hydrophobicity over this log K_{ow} range. DT_{95} values, however, do reflect some dependence on hydrophobicity. Esfenvalerate and chlorpyrifos had DT_{95} values of 380 and 400 days, respectively, whereas DT_{95} values for diflubenzuron were 27 and 45 days for applications 1 and 2, respectively. The DT_{95} value for azinphos-methyl was 30 days.

Macrophyte Residues. *Low Concentration (2.5 μg/L).* Diflubenzuron residues in the macrophytes on a dry weight basis were 3–4 orders of magnitude greater than in the sediment. Diflubenzuron residues measured 254 μg/kg 1 day after application 1 and reached a maximum of 332 μg/kg on day 4 (Figure 3). The residue level dropped below detection (<2.94 μg/kg) at 14 days and was 159 μg/kg 32 days after application 1. After application 2, greater amounts were detected at 3 h, 2 days, and 14 days with values of 1320, 2480, and 1470 μg/kg, respectively. The residue levels fluctuated over a large range with no consistent trend apparent. Diflubenzuron was not detected (<2.94 μg/kg) in the macrophytes 32 and 56 days after application 2.

High Concentration (30 μg/L). Diflubenzuron residues in the macrophytes of the high-treatment enclosure were the highest of any component measured (Figure 3). Residue concentration after application 1 reached a maximum of 6420 μg/kg on day 4. Residue levels were somewhat higher after application 2 with values of 27 000 μg/kg after 3 h and 48 500 μg/kg after 1 day. The residue levels in the macrophytes from this study compare well with those of Booth and Ferrell (1977). They noted that diflubenzuron residues in aquatic vegetation were the highest of all compartments studied in their aquatic system. They measured a maximum

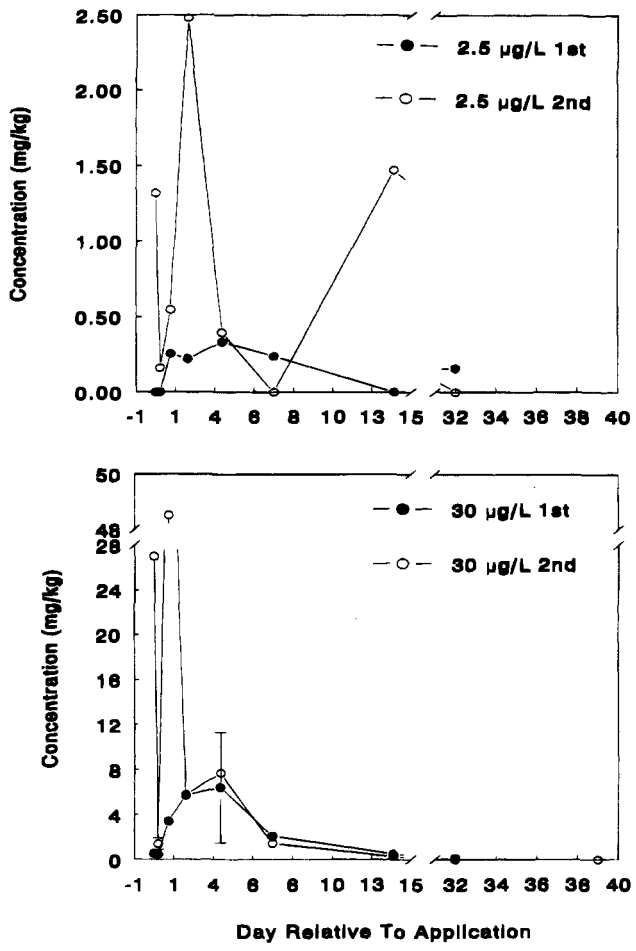

Figure 3. Diflubenzuron residue concentrations associated with the macrophytes of 2.5 and 30 µg/L littoral enclosures. Data where error bars are present are means of two duplicate samples collected at the same time from the same enclosure. Error bars indicate one standard deviation about the mean (n = 2).

value of 9300 µg/kg 7 days after the first of four spray applications but also noted that residues in aquatic vegetation dropped to below detection (50 µg/kg) within 28 days of their final application of diflubenzuron. Sundaram et al. (1991) reported aquatic vegetation residue values of up to 360 µg/kg after aerial application to a forest aquatic ecosystem. They reported a persistence of diflubenzuron in aquatic vegetation of 7–10 days, which was 10 days less than in the water. No DT_{50} or DT_{95} values were estimated in these other studies.

In this littoral enclosure study, diflubenzuron persistence in the macrophytes after application 1 agreed very well with the observed sediment persistence. Diflubenzuron persistence in the macrophytes was not as great after the second application, which is the inverse of the trend seen in the sediment. DT_{50} values were 5.6 and 2.0 days for applications 1 and 2, respectively. DT_{95} values were 24.6 and 8.6 days for applications 1 and 2, respectively (Table 7). The greater rate of degradation after application 2 was probably due to the very large residue value observed 1 day after application 2. This large value probably influences the slope of the line resulting in a greater calculated loss rate. The data for 2–14 days for each application are very similar, suggesting that the degradation rates were probably more similar than different.

Distribution of Diflubenzuron. *Low Concentration (2.5 µg/L).* The aqueous phase contained the most

Table 7. Estimated DT_{50} and DT_{95}[a] Values for Diflubenzuron Associated with Littoral Enclosure Macrophytes

2.5 µg/L treatment level	equation[b]	r^2	DT_{50} (days)	DT_{95} (days)
application 1	$\ln c = 8.61 - 0.122t$	0.876	5.7	24.6
application 2	$\ln c = 10.2 - 0.348t$	0.874	2.0	8.6

[a] Time (days) for 50% and 95% dissipation, respectively. [b] From linear regression of ln concentration (µg/kg) vs time (days) (application 1, n = 4; application 2, n = 5).

diflubenzuron, on a mass per enclosure basis, of all components measured, particularly after application 1. The maximum amount accounted for in the aqueous phase was 82.3% 3 h after application 1 (Table 8). The amount steadily decreased to 32.8% 2 days after application 1, and none was detected in the water (<0.12 µg/L) after 7 days. The sediment contained amounts ranging from 3.2 to 1.8% between 1 and 7 days after application 1. Amounts in the macrophytes ranged from 0.6 to 0.9% between 1 and 7 days after application 1.

After application 2, the water was again the dominant component for diflubenzuron mass distribution. However, the overall amount measured was less than for all other applications, particularly after 1 and 2 days, when the water accounted for only 36.0% of the applied diflubenzuron. The maximum of 38.7% occurred after 2 days, and the last measured amount on day 14 accounted for 11.1%. The amount measured in the sediment was similar to that measured after the first application. The maximum amount of 2.7% was measured 9 h after application 2, with the last quantifiable amount of 1.4% occurring 7 days after application 2. The macrophytes contained more measurable diflubenzuron after application 2 than after application 1, with a maximum contribution of 7.7% occurring 2 days after application (Table 8).

High Concentration (30 µg/L). The aqueous phase of the littoral enclosures was again the most significant component for diflubenzuron, on a mass per enclosure basis, after each application. After application 1, the water accounted for 52.8% of the applied chemical after 3 h, compared to 0.3% for sediment and 0.1% for macrophytes (Table 9). The macrophytes had the highest concentrations on a per mass basis, but the overall biomass present in the enclosures limits the contribution of macrophytes in the mass distribution of diflubenzuron. By 32 days after application 1, the water accounted for none of the diflubenzuron applied, the sediment for 0.2%, and the macrophytes for <0.1%. The maximum amount of diflubenzuron accounted for by the sediment was observed 4 days after application 1 (6.3%). The macrophytes accounted for a maximum of 1.2% 4 days after application 1.

After application 2, the same distribution pattern was observed as after application 1. The maximum amount accounted for in the aqueous phase was 44.0% 3 h after application, and the contribution by the water decreased to 1.8, 0.5, and 0.0% after 14, 32, and 56 days, respectively (Table 9). The maximum observed in the sediment according to distribution was 7.4% 2 days after application 2. For the macrophytes, the maximum amount of 10.2% was observed 1 day after application 2. By 56 days after application 2, all compartments of the littoral enclosures were below their respective detection limits (Table 2).

Mass Balance. *Low Concentration (2.5 µg/L).* The mass balance of diflubenzuron in the 2.5 µg/L enclosure

Table 8. Diflubenzuron Distribution and Mass Balance in a 2.5 μg/L Littoral Enclosure

time[a]	amt applied (mg)	amt detected (mg/enclosure)				amt detected (% of applied)			
		W[b]	S[c]	M[d]	sum	W	S	M	sum
0 h	75.0								
3 h		61.7	ND[e]	ND	61.7	82.3	0.00	0.00	82.3
9 h		45.2	ND	ND	45.2	60.3	0.00	0.00	60.3
1 day		40.1	2.11	0.51	42.8	53.5	2.81	0.69	57.0
2 days		24.6	2.09	0.45	27.1	32.8	2.78	0.59	36.1
4 days		15.0	1.37	0.67	18.0	20.0	3.18	0.90	24.1
7 days		8.69	ND	0.48	10.5	11.6	1.83	0.64	14.1
14 days		ND	ND	ND	ND	0.00	0.00	0.00	0.00
32 days		ND	ND	0.32	0.32	0.00	0.00	0.43	0.43
32 days [0 h]	65.0								
33 days [3 h]		23.4	ND	2.67	26.0	36.0	0.00	4.12	40.1
33 days [9 h]		23.4	1.72	0.33	25.4	36.0	2.65	0.50	39.1
34 days [1 day]		20.0	0.65	1.10	21.8	30.8	1.00	1.69	33.5
35 days [2 days]		25.2	1.43	5.01	31.6	38.7	2.20	7.71	48.6
37 days [4 days]		16.4	1.53	0.80	18.8	25.3	2.35	1.23	28.9
40 days [7 days]		14.4	0.93	ND	15.3	22.1	1.43	0.00	23.6
47 days [14 days]		7.19	ND	2.97	10.2	11.1	0.00	4.57	15.6
71 days [32 days]		NS[f]	NA[g]	ND	ND	0.00	0.00	0.00	0.00
85 days [56 days]		NS	NA	ND	ND	0.00	0.00	0.00	0.00

[a] Time relative to application 1 [application 2]. [b] Water. [c] Sediment. [d] Macrophytes. [e] None detected. [f] Not sampled. [g] Not analyzed.

Table 9. Diflubenzuron Distribution and Mass Balance in a 30 μg/L Littoral Enclosure

time[a]	amt applied (mg)	amt detected (mg/enclosure)				amt detected (% of applied)			
		W[b]	S[c]	M[d]	sum	W	S	M	sum
0 h	525								
3 h		277	1.61	0.55	280	52.8	0.31	0.11	53.3
9 h		216	1.35	0.48	218	41.1	0.26	0.09	41.5
1 day		218	24.1	3.28	245	41.5	4.60	0.62	46.7
2 days		183	28.5	5.49	217	34.8	5.42	1.05	41.3
4 days		NS[e]	33.1	6.16	39.3	NS	6.31	1.17	7.48
7 days		93.4	28.2	2.04	124	17.8	5.38	0.39	23.6
14 days		25.3	6.63	0.45	32.4	4.82	1.26	0.09	6.17
32 days		ND[f]	1.18	0.14	1.32	0.00	0.23	0.03	0.25
33 days [0 h]	456								
33 days [3 h]		201	0.39	25.9	227	44.0	0.09	5.68	49.8
33 days [9 h]		195	20.0	1.37	216	42.7	4.38	0.30	47.4
34 days [1 day]		181	17.9	46.6	246	39.7	3.91	10.2	53.8
35 days [2 days]		181	33.9	5.53	221	39.7	7.42	1.21	48.3
37 days [4 days]		144	9.37	7.38	161	31.5	2.05	1.62	35.2
40 days [7 days]		48.3	9.08	1.40	58.8	10.6	1.99	0.31	12.9
47 days [14 days]		8.07	6.64	0.26	15.0	1.77	1.45	0.06	3.28
71 days [32 days]		2.13	3.30	ND	5.43	0.47	0.72	0.00	1.19
85 days [56 days]		NS	ND	ND	ND	0.00	0.00	0.00	0.00

[a] Time relative to application 1 [application 2]. [b] Water. [c] Sediment. [d] Macrophyte. [e] Not sampled. [f] None detected.

was similar to that in the 30 μg/L enclosure, with the overall mass balance reflecting the amount measured in the water. For the 2.5 μg/L enclosure, the mass balance reached a maximum of 82.3% 3 h after application 1, which was the maximum value observed for the distribution and mass balance study. The amount decreased to 0.4% by 32 days after application 1. The maximum amount accounted for 2 days after application 2 was 48.6%, and this amount decreased to 15.6% 14 days after application (Table 8).

High Concentration (30 μg/L). The mass balance of diflubenzuron reached a maximum of 53.3% 3 h after application 1 and remained slightly below this level through 2 days, when 41.3% of the diflubenzuron applied was accounted for. By 32 days after application 1, the mass balance was 0.3%. A similar pattern was observed after application 2, with the maximum of 53.8% observed 1 day after application. The mass balance once again remained fairly constant throughout the first 2 days after application 2. By 32 days after application 2, 1.2% of the amount applied was accounted for and none was present in any component of the littoral enclosures 56 days after application 2 (Table 9).

No other studies appear to have specifically determined the mass balance of diflubenzuron after application to an aquatic system. However, the results of this study are similar to other studies in that diflubenzuron normally persists for 2–3 days in the water and limited persistence in sediment and aquatic vegetation has been noted (Apperson et al., 1978; Booth and Ferrell, 1977; Schaefer and Dupras, 1976; Sundaram et al., 1991). Longer persistence in water of up to 7 weeks has been observed in aquatic systems of low pH (Ivie et al., 1980).

With respect to previous littoral enclosure studies with other pesticides, some general trends are apparent. As the log K_{ow} of the chemical increases, the half-life in the water column decreases and sorption to compartments such as sediment and aquatic vegetation increases, as does the persistence of the chemicals there. However, this increase in sorption and persistence is offset by a decrease in the mass balance of the chemical as the log K_{ow} increases. This effect may be due to increased irreversible sorption to organic matter as log K_{ow} increases or to more rapid degradation, particularly in the water, as a function of log K_{ow}.

CONCLUSIONS

Maximum residues of diflubenzuron in the water column were measured within the first 24 h following application. The average losses of residue between 1 and 24 h was 34 and 37% after two subsequent applications 32 days apart. Diflubenzuron residues in the 0.7 and 30 μg/L treatments remained detectable for approximately 7–32 days, respectively. Water column half-lives ranged from 3.3 to 8.2 days, with a mean of 4.3 days, and it took 14–35 days for 95% of the residue to dissipate.

The precision of the measured concentrations at each respective treatment level was comparable to that of other pesticides studied in the littoral enclosures. The overall coefficient of variation for diflubenzuron was 23.2% ($n = 41$). Measured concentrations for all enclosures following both treatments were an average of 21% below the nominal target concentrations. The nominal and measured concentrations had a general tendency to agree better in the lower treatments. Analysis of select enclosures revealed that concentrations in the water column remained vertically nonhomogeneous at 1 and 9 h but were homogeneous by 24 h.

The water was the major compartment for diflubenzuron during the first 7 days after application, accounting for a maximum of 82.3% of that applied after 3 h and 11.6% after 7 days. Sorption to the sediment and macrophytes accounted for maximum values of 6.3 and 10.2% of that applied, respectively. The overall mass balance of diflubenzuron ranged from 82.3% of the applied chemical after 3 h to nondetectable after 56 days.

The water appears to be the major route of exposure of planktonic and free-swimming aquatic organisms to diflubenzuron. The macrophyte community may also provide an exposure route to those organisms dwelling within the macrophytes and grazing upon them. The sediment would probably play only a minor role in exposure of aquatic organisms dwelling in the overlying water but would be the principal route of exposure for those organisms dwelling in the sediment, particularly the detritivores.

ACKNOWLEDGMENT

This research was a cooperative effort between the U.S. Environmental Protection Agency and the University of Wisconsin—Superior conducted under Agreement CR-816758. We thank Richard Siefert, project manager, and Karsten Liber, principal investigator, for project direction and advice. We gratefully acknowledge the work of Thomas Melvin and Renee Tunell, who assisted in the pesticide application, sample collection, extraction, and analysis and prepared the tables and figures for the manuscript, and Lee Anderson, who performed the water quality parameter analysis and interpretation. We also thank the city of Duluth, MN, for use of the study site.

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Received for review July 18, 1994. Revised manuscript received December 6, 1994. Accepted January 30, 1995.[⊗]

JF9404004

[⊗] Abstract published in *Advance ACS Abstracts,* March 1, 1995.