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Assessment of Diazinon Toxicity in Sediment and Water of Constructed Wetlands Using Deployed *Corbicula fluminea* and Laboratory Testing

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Abstract. Constructed wetlands for mitigation of nonpoint agricultural runoff have been assessed for their ability to decrease potential toxicity from associated contaminants. After a simulated runoff event, constructed wetlands positioned in series were used to measure the effects of the organophosphate insecticide diazinon. Water, sediment, and plant samples from five sites were analyzed for diazinon concentrations from 0.5 hours to 26 days; peak concentrations were measured in sediment after 0.5 hours (268.7 μg/kg) and in water and plant tissue after 3 hours (121.71 μg/L and 300.7 μg/kg, respectively). Cholinesterase activity and changes in shell growth were measured from *Corbicula fluminea* deployed at corresponding sites. Water collected after 9 hours from all wetland sites contained diazinon concentrations sufficient to cause toxicity to *Ceriodaphnia dubia*, but not to *Pimephales promelas*. *C. dubia* survival was decreased in water sampled through 7 days from the site nearest runoff introduction, whereas *C. fluminea* deployed at this same site experienced 100% mortality after 26 days. Clams from lower sites survived wetland conditions, but growth and ChE activity were significantly decreased lower than that of clams from a control site. *C. dubia* exposed to water from these sites continued to have decreased survival throughout the 26-day sampling. Sediment sampled from 48 hours through 14 days at the lowest wetland site decreased the laboratory survival of *Chironomus dilutus*, and sediment from upper sites elicited an effect only on day 26. Although wetland concentrations of aqueous diazinon were decreased lower than toxic thresholds after 26 days, decreased ChE activity in deployed clams provided evidence of residual diazinon effects to deployed organisms.

Concentrations of pesticides leaving agricultural fields are in part determined by the efficacy of implemented agricultural best management practices (BMPs). Practices that include buffer strips, constructed wetlands, and vegetated drainage ditches have been shown to decrease contaminants associated with agricultural runoff (De Laney 1995; Cooper *et al*. 2004). Chemical properties of the pesticide, and the physical, chemical, and biologic characteristics of the receiving systems, combine to enhance mitigation of agricultural-associated contaminants. Attributes of these systems that modify runoff include vegetative and sediment interactions, residence time, and spatial distance from point of input (Bouldin *et al*. 2004a). Macrophyte communities act as a sink for runoff-associated pesticides and also increase hydraulic retention time in agricultural receiving systems (Runes *et al*. 2001; Schulz *et al*. 2003). The importance of retention time has been noted for the uptake rates of various pesticides (Lytle & Lytle 2002) and has been illustrated in studies addressing vegetated agricultural drainage ditches and constructed wetland cells (Bouldin *et al*. 2004a, 2004b; Milam *et al*. 2004). Vertical wetlands, as described by De Laney (1995), serve as ecotones by providing a spatial and temporal distance between agricultural fields and receiving streams through hydraulic retention inherent with their successive structure and macrophyte presence.

Wetland hydrology, structural attributes, and chemical properties have been used to model the rate of pesticide transfer and transformation in constructed wetlands (Kadlec & Knight 1996). The ability to mitigate nontarget effects is a product of pesticide removal rate and residence time in a wetland and has been modeled as a function of contact time in the wetland and macrophyte sorption qualities of biotransformation and photolysis (Rodgers & Dunn 1992). In addition, phytoaccumulation, phytoextraction, phytovolatilization, and rhizodegradation from enhanced microbial activity in plant rhizospheres are known phytoremediation pathways (Mirgain *et al*. 1993; Susurla *et al*. 2002; Walton & Anderson 1990).

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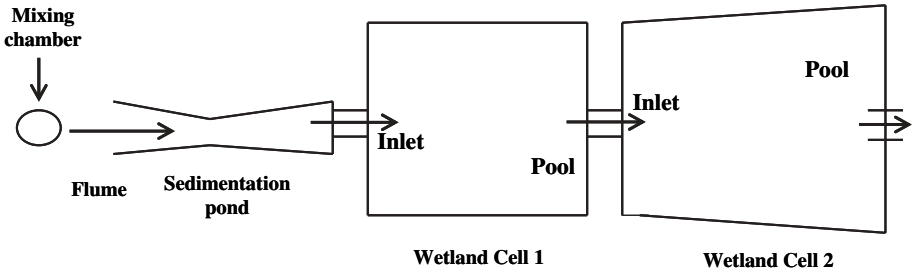

Fig. 1. Schematic drawing constructed wetland cells, clam deployment and sampling sites, and hydrologic flow for diazinon exposure

Although models, such as those described by Rodgers and Dunn (1992), provide useful predictions of pesticide fate in constructed wetlands, complex interactions among attributes dictate the need for field studies to validate actual fate of agricultural-associated pesticides entering these systems.

Many field simulations of pesticide exposure, such as constructed wetlands and agricultural ditches, fail to include systems that allow uptake and transformation of contaminants from production fields. Integrating receiving systems with deployed organisms will incorporate ambient site conditions (*e.g.*, macrophyte and sediment interaction, landscape features, chemical parameters) that are often overlooked for hazard assessment in agricultural landscapes. Such assessments of water and sediment toxicity from nonpoint source contaminants have successfully incorporated standard test organisms placed *in situ* to provide ecologically relevant toxicity measurements (Schulz & Liess 1999; Phillips *et al*. 2004). Although sublethal responses of nontarget organisms to pesticides have been investigated, assessment of pesticide mitigation on such end points has not typically been included. Standardized laboratory testing, coupled with biomarkers from deployed organisms, may provide a measure of mitigation capability of agricultural receiving areas, such as constructed wetlands. In addition, they offer a contrast of survival end points coupled to continuous exposure of organisms *in situ*.

This field study examined the effects of the organophosphate insecticide, diazinon [O,O-diethyl 0-2-isopropyl-6-methyl (pyrimidine-4-yl) phosphorothioate], in a constructed wetland in the Mississippi Delta. Its prevalence in agricultural soils, ditch water and sediments, and agricultural receiving streams (Wan *et al*. 1994) occurs because of its environmental persistence. Diazinon's water solubility (60 mg/L), hydrolysis half-life (138 days), moderate toxicity potential to aquatic organisms (http://www.arsusda.gov/acsl/services/ppdb/), use exceeding 258,500 kg/y (http://www.pestmanagement.info/nass), and detectable concentrations of diazinon in receiving systems (Katznelson & Mumley 1997) validates the need for field monitoring. Diazinon is a restricted-use pesticide and is currently under study for use in fire ant control in the Mississippi Delta agricultural landscape. Decreased fish and invertebrate populations as well as regionally measured diazinon concentrations exceeding state water-quality guidelines have prompted similar studies in California's Central Valley (Joyce *et al*. 2004). This study attempted to elucidate the effects of diazinon on laboratory test organisms and deployed clams using toxicologic end points from acute aqueous and sediment tests concurrent with biomarker measurements from deployed clams. Simultaneous measurements of test organism survival under laboratory conditions were compared with responses of deployed clams exposed to wetland conditions. These end points were used to determine the efficacy of pesticide mitigation offered by constructed wetlands receiving agricultural runoff.

Materials and Methods

Constructed wetlands, established in April 2003, consisted of a sedimentation pond and two wetland cells in series (24 × 22 m and 28 × 22 m) with a combined hydrologic capacity of 1.12 million L (Fig. 1). Wetlands were used to capture runoff from a simulated rainfall event on an agricultural field after application of the organophosphate insecticide, diazinon, as Diazinon 4E. The wetland system received no previous agricultural runoff, which contributed to its ability for use in controlled studies. This simulated rainfall event was used to determine acute effects measured in standard test organisms exposed to sampled sediments and water. In addition, *Corbicula fluminea* (Asian clam) were placed at sampling sites 24 hours before dosage and retrieved at timed intervals. End points measured for deployed organisms were survival, cholinesterase (ChE) activity, and growth. Ninety-five percent vegetative cover within the wetland included plant communities of 85% *Alternanthera* sp., 10% *Leersia* sp., and 5% *Rumex crispus*.

A 3785-L chamber was used to mix 660 mL Diazinon 4E with 3785 L water from an adjacent oxbow lake. The addition of sediment to the mixing chamber achieved a target concentration of 394 mg/kg as calculated from an estimated 0.394 g dry sediment/L runoff to simulate suspended sediment typically found in field runoff (Smith *et al*. 2002). The pesticide–sediment mixture from the mixing chamber was combined with water from the oxbow lake just before passing through a weir and flowing into the sedimentation pond and wetland series (Fig. 1). Source water used to simulate runoff had no detectable diazinon concentrations (Cooper *et al*. 2003). A 1% diazinon runoff was calculated using the recommended field application rate and a 1.3-cm storm event on a 14.16 ha contributing field, resulting in 917,335 L water to simulate the runoff. Before runoff simulation, wetland cells were flooded to capacity, resulting in almost complete hydrologic turnover during the event. Reported sampling sites included the sedimentation pond, inlet, and downstream in the first wetland cell (cell 1 intake and cell 1 pool, respectively); inlet and downstream in second wetland cell (cell 2 intake and cell 2 pool, respectively); and source water site in the oxbow lake (lake site). Aqueous grab samples were collected for chemistry and toxicity by immersing sampling containers; collecting sediment from only the top 3 cm with Teflon spatulas; and extracting plant tissue from the plant–aqueous interface. Sampling was accessed from walkways established within the wetlands.

Physicochemical Parameters

Water-quality parameters were measured at sampling locations to verify comparable exposure conditions of deployed organisms.

Temperature, pH, dissolved oxygen (DO), and conductivity were measured at the sedimentation pond, cell 1 intake, cell 1 pool, and cell 2 intake at 0 hours and at 7, 14, and 26 days after the simulated runoff event. At cell 2 pool and lake site, these parameters were measured at 0, 9, 24, 48 hours and at 7, 14, and 26 days after the runoff event. In addition, samples collected at comparable depths from cell 2 pool and lake site were returned to the laboratory on ice and analyzed for carbonate alkalinity, hardness, nitrate, nitrite, phosphorus as orthophosphate, chlorophyll *a*, fecal coliforms, and total, suspended, and dissolved solids. Water-quality measurements were taken on unfiltered water according to the American Public Health Association (APHA 1998) methods using a YSI Model 610 multimeter (Yellow Springs, OH) for DO and temperature; an Accumet AR 25 dual channel pH and ammonia meter (Fisher Scientific, Houston, TX) for pH determination; and a Hach DR 890 colorimeter (Hach Company, Loveland, CO) for nutrient analyses. Nitrate (NO_3^-) determinations were made according to the cadmium reduction and diazotization method with a 0.01 mg/L detection limit. Nitrite (NO_2^-) determinations were made according to the diazotization method with a 0.005 mg/L detection limit. Total reactive phosphorus (PO_4^{3-}) determinations were made according to the ascorbic acid method with a 0.05 mg/L detection limit. Chlorophyll *a* concentrations were determined with a Beckman DU 640 spectrophotometer according to APHA (1998) sample preparation methods. Microbial examination included enumeration of blue colonies for positive fecal coliform identification.

Pesticide Analyses

Pesticide analyses were conducted on unfiltered water, sediments, and plants by way of a method similar to that of Bennett *et al*. (2000) and modified by Smith and Cooper (2004). This involved extraction with pesticide-grade ethyl acetate, silica gel column chromatography cleanup, and concentration to 1 mL volume under high-purity dry nitrogen. Recoveries based on fortified samples (five replicates each at fortification levels of 0.01, 0.1, and 1.0 µg/L for water and 0.01, 0.1, and 1.0 µg/kg for sediments and plants) were <90%. Two Hewlett Packard (now Agilent) model 6890 gas chromatographs, each equipped with dual HP 7683 ALS autoinjectors, dual split-splitless inlets, dual capillary columns, an HP Kayak XA Chemstation were used to conduct all pesticide analyses (Smith & Cooper 2004). One HP 6890 was equipped with two HP microelectron-capture detectors (µECDs) and the other 6890 with one HP µECD, one HP nitrogen–phosphorus detector, and an HP 5973 mass selective detector. The main analytic column was an HP 1MS capillary column (30 m × 0.25 mm i.d. × 0.25 µm film thickness). Column oven temperatures were as follows: initial at 85°C for 1 minute, ramp at 25°C/min to 185°C, and hold at 185°C for 20 minutes for a total runtime of 25 minutes. The carrier gas was ultra high purity (UHP) helium at 24 cm/s average velocity with the inlet pressure at 11.99 psi and inlet temperature at 250°C. The µECD temperature was 325°C with a constant makeup gas flow of 60 cc/min UHP nitrogen. The autoinjector was set at 1.0 µL injection volume in the fast mode. Under these GC conditions, retention time for diazinon was 10.852 minutes. The limit of quantification (LOQ) for this analysis was 0.1 µg/L and the limit of detection (LOD) 0.01 µg/L for diazinon in aqueous samples. Using control samples, the LOD was determined as the average noise of the chromatographic baseline plus three times the SD of this value. The LOQ was taken as 10 times the LOD.

Before test initiation, analyses were conducted on water, sediment, and plant material extracted from the sedimentation pond, cell 1 pool, and cell 2 pool to determine background diazinon levels. Chemical analyses for diazinon were conducted on water and sediment extracted from the sedimentation pond, cell 1 intake, cell 1 pool, cell 2 intake, and cell 2 pool at 0.5, 3, 9, 24, 48 hours and at 7, 14, and 26 days and on plant material from the sedimentation pond, cell 1 intake, cell 1 pool, and cell 2 intake at 0.5, 3, 9, 24, 48 hours and 7, 14, and 26 days. In addition, plant material was analyzed at 3, 9, 24, 48 hours and 7, 14, and 26 days from cell 2 pool.

Toxicity Testing

Forty-eight hour acute toxicity in aqueous samples was assessed with *Ceriodaphnia dubia* (water flea) and *Pimephales promelas* (fathead minnow) using standardized methods (United States Environmental Protection Agency [USEPA] 2002). Inhibition of *Chironomus dilutus* (midge larvae) survival and growth was assessed in solid-phase 10-day sediment tests (USEPA 2000). Fresh overlying water was furnished to test chambers twice daily using a static renewal system as dechlorinated Jonesboro, AR, municipal tap water. In addition, organisms in each test chamber were fed 1 ml Tetramin solution (4 g/L) daily during the 10 days. Water quality was measured at regular intervals in randomly selected test chambers for each of the sites and included temperature (°C), DO (mg/L), conductivity (µS/cm), and pH (APHA 1998). Toxicity assay results were analyzed statistically using Toxcalc (version 5.0.25) (McKinleyville, CA). Normality assumptions were tested using Shapiro-Wilk's test and Steel's Many-One Rank test to compare variation in survival among sites ($\alpha = 0.05$).

Aqueous toxicity assessments were conducted on samples collected from the sedimentation pond, cell 1 intake, cell 1 pool, cell 2 intake and lake site at 0, 0.5, 3, 9, 24, 48 hours and at 7, 14, and 26 days. In addition, samples from cell 2 pool were also evaluated for aqueous toxicity at 0, 3, 9, 24, and 48 hours and at 7, 14, and 26 days. Solid-phase 10-day acute testing was performed on sediments from the sedimentation pond, cell 1 intake, cell 1 pool and, cell 2 intake at 0, 0.5, 3, 9, 24, and 48 hours and at 7, 14, and 26 days. In addition, biomonitoring was performed on sediments from cell 2 pool at 0, 3, 9, 24, and 48 hours and at 7, 14, and 26 days and from lake site at 0 hours and at 7, 14, and 26 days.

In Situ Biomarkers

C. fluminea were collected from the Strawberry River near Ash Flat, Sharp County, AR, at a reference site with no upstream industrial discharges. Specimens were collected 3 days before deployment and transported to Arkansas State University Ecotoxicology Research Facility where they were placed in flow-through holding tanks filled with dechlorinated municipal tap water (Jonesboro, AR, United States). Before placement, clams were enumerated and permanently identified with a Dremel tool for shell growth measurement. Clams were placed into 6 mesh bags (84 clams/bag for the sedimentation pond and 72 clams/bag for remaining sites) and a mesh bag introduced to each sampling site 24 hours before dosage. Biomarkers were measured from the sedimentation pond at 3, 9, 24, and 48 hours and at 7, 14, and 26 days, whereas biomarkers were measured from cell 1 intake, cell 1 pool, cell 2 intake, cell 2 pool, and lake site at 9, 24, and 48 hours and at 7, 14, and 26 days. In addition, shell growth was obtained from killed organisms at 7, 14, and 26 days as the maximum anteroposterior dimension. Survival was assessed through responsiveness to physical stimulus, gaping, and eviscerated tissue. Clams not sampled for biomarker measurements remained in bags and were returned to exposure sites.

Statistical Analysis

Results from growth observations were analyzed using one-way analysis of variance techniques. All tests for significance were conducted using $\alpha = 0.05$. For all analyses, data were tested for normality

using Kolmogorov-Smirnov statistic and for homogeneity of variance using Bartlett's test (Minitab 2000; State College, PA).

Tissue Collection and Preparation for ChE

Anterior adductor muscles of *C. fluminea* were dissected and flash frozen on site to determine ChE activity. This tissue has been shown to contain greater levels of ChE than other tissues in the clam (Moulton *et al*. 1996), facilitating its use as an enzyme source for the bioassay. On return to the laboratory, samples were stored at −80°C before analyses.

Individual adductor muscle samples were homogenized for 30 to 40 seconds with 1:50 w/v 0.05 M Tris buffer at pH 8.0 and 1% Triton X-100 detergent using a Tekmar TP/10S1 tissue homogenizer (Winchester 1997). Samples were centrifuged at 10,000 × *g* for 10 minutes at 4.0°C, and the supernatant was collected and maintained on ice (approximately 4.0°C) until assayed.

ChE Assay and Calculations

Total adductor-muscle ChE activity was measured colorimetrically with a modification of the methods of Ellman *et al*. (1961) using a 96-well microplate spectrophotometer (Tecan Austria GmbH, Grödig/Salzburg, Austria) in kinetics mode at 25°C (Winchester 1997). Absorbance readings were taken every 10 s for 5.83 minutes at λ = 412 nm. Acetylthiocholine iodide (AThCh) was used as a substrate for the cholinesterase enzyme and enzyme activity was expressed as nanomoles of AThCh hydrolyzed/min/mg protein. The following reagent volumes and final concentrations were added to appropriate microplate wells for the assay: (1) 5',5'-dithiobis-(2-nitrobenzoic acid) (3.19×10^{-4} M); (2) 0.05 M Tris buffer at pH 8.0 to blank wells; (3) aliquot of diluted (50-fold) and vortexed tissue to sample wells; and (4) AThCh (final concentration = 2.44×10^{-4} M) to all wells. The final assay volume was 200 μL/microplate well. Standards and blanks were run simultaneously to samples for each plate. All chemicals were obtained from Sigma Chemical Company (St. Louis, MO).

Analyses were performed in triplicate and data was standardized by expressing ChE as a percent ChE activity of organisms retrieved concurrently from the lake site (control site) and assayed within the same run as a control. Additional controls from clams killed immediately after collection from the Strawberry River, AR, had measured ChE activities with no statistical differences to clams retrieved from the Lake Site.

Protein Assay

Adductor-muscle supernatant was diluted 100-fold and protein concentration was determined using the Bio-Rad Detergent Compatible protein assay, which is a modification of the Lowry method. The protein assay was performed using a 96-well microplate spectrophotometer (Tecan Austria GmbH, Grödig/Salzburg, Austria) taking five single absorbance readings every 10 seconds at λ = 750 nm at 25°C with bovine gamma globulin as protein standard.

Results

Physicochemical Parameters

Throughout the exposure, conductivity, carbonate alkalinity, and hardness were slightly higher in water from the lake site than from cell 2 pool (Table 1). Higher mean total suspended solids (TSS) (mean TSS = 53.8 and 33.6 mg/L) were measured in water from the lake site with significantly higher values in water sampled at 0 and 48 hours (113 and 60 mg/L and 123 and 7 mg/L, respectively). Chlorophyll *a* values were higher in water extracted from cell 2 pool (chlorophyll *a* = 19.65 and 53.40 μg/L, respectively). Water-quality parameters for remaining wetland sites were comparable with measured parameters from cell 2 pool.

Table 1. Measured physicochemical parameters from lake site and cell 2 pool sites for May through June 2003[a]

Site	*n*	Lake site	Cell 2 pool
pH	4	7.17 ± 0.52	7.23 ± 0.68
Temperature (°C)	4	25.4 ± 1.2	25.1 ± 1.2
DO (mg/L)	4	5.9 ± 1.5	5.1 ± 2.2
Conductivity (μS/cm)	4	61.6 ± 2.2	41.4 ± 11.4
Carbonate alkalinity (mg/L)	4	31 ± 5	24 ± 10
Hardness (mg/L)	4	30 ± 4	25 ± 6
TSS (mg/L)	7	53.8 ± 46.9	33.6 ± 23.1
Turbidity (NTU)	7	36.0 ± 15.9	17.8 ± 8.7
Chlorophyll *a* (mg/L)	7	19.65 ± 11.27	53.40 ± 30.42
Fecal coliforms (CFU/100 mL)	7	231 ± 428	100 ± 140
Nitrate (mg/L)	7	0.04 ± 0.02	0.02 ± 0.02
Nitrite (mg/L)	7	0.011 ± 0.007	0.013 ±0.012
Orthophosphate (mg/L)	7	0.55 ± 0.14	0.61 ± 0.33

CFU = Colony forming unit; NTU = Nephelometric turbidity.
[a] Means ± 1 SD provided.

Pesticide Analyses

Diazinon was detected in water from the first wetland cell 0.5 hours after dosage and from all wetland sites within 3 hours (Table 2). Measurable pesticide concentrations (0.06 μg/L) persisted in water from the downstream wetland cell (cell 2 inlet and cell 2 pool) through 26 days. Although the highest aqueous concentration was detected at 3 hours from cell 2 inlet (182.16 μg/L), the highest concentration in sediment was measured 0.5 hours after introduction of diazinon from cell 1 inlet (268.7 μg/kg). Peak diazinon concentration in plant tissue was also measured at 3 hours from cell 1 pool (300.7 μg/kg). Detectable concentrations remained in plant tissue extracted from the second wetland cell throughout the 26-day exposure.

Toxicity Testing

Although *P. promelas* survival was not decreased in water from wetland sites throughout the exposure, diazinon transport was indicated by increased toxicity responses of *C. dubia* in water from sites through the wetland (Table 3). Although *C. dubia* failed to survive in water sampled at 0.5 hours from sedimentation pond and cell 1 inlet, partial survival was measured in water collected at this time from cell 1 pool and cell 2 inlet (20% and 65%, respectively). *C. dubia* survival was significantly decreased (≤ 5%) in water collected at 3 hours from the sedimentation pond, cell 1 inlet, cell 1 pool, and cell 2 inlet. Although *C. dubia* survived exposure to water collected from cell 2 pool at this time, toxicity was measured in subsequent

Table 2. Measured pesticide concentrations from constructed wetlands after application in 2003[a]

Location and type		Time postapplication								
		0 h	0.5 h	3 h	9 h	24 h	48 h	7 d	14 d	26 d
Sedimentation pond	Water	ND	102.45	95.41	14.85	11.46	0.18	1.10	0.16	ND
	Sediment	ND	ND	ND	16.5	ND	ND	5.5	ND	ND
	Plant	NS	NS	NS	198.4	NS	172.1	168.9	ND	ND
Cell 1 inlet	Water	NS	91.69	106.63	14.07	21.14	ND	0.08	1.70	ND
	Sediment	NS	268.7	ND	ND	1.3	ND	ND	44.8	ND
	Plant	NS	90.4	101.4	147.6	41.5	66.9	43.6	8.8	ND
Cell 1 pool	Water	0.55	104.57	121.71	0.06	47.88	1.41	ND	0.43	ND
	Sediment	32.8	ND	ND	ND	ND	18.6	ND	ND	NS
	Plant	4.8	75.4	300.7	73.5	97.6	102.2	ND	20.2	ND
Cell 2 inlet	Water	NS	ND	182.16	ND	0.02	ND	0.05	4.80	0.06
	Sediment	NS	ND	ND	ND	1.9	14.8	99.0	10.4	99.0
	Plant	NS	ND	84.7	81.5	65.4	108.6	112.6	47.1	23.9
Cell 2 pool	Water	0.08	ND	0.07	19.26	45.96	ND	ND	0.19	0.06
	Sediment	11.4	ND	23.8	ND	ND	12.4	3.5	ND	ND
	Plant	33.7	NS	140.1	ND	126.1	135.0	88.9	172.3	40.3

NS = not sampled for GC analysis; ND = not detected (lower than LOD).
[a] LOD for diazinon in water, sediments, and plants = 0.01 μg/L, 0.1 μg/kg, and 0.1 μg/kg, respectively.

samples from this site. *C. dubia* survived exposure to water collected at 14 days from the sedimentation pond, cell 1 inlet, cell 1 pool, and cell 2 inlet, and although these organisms survived in water collected at 26 days from the sedimentation pond, survival was decreased in water from cell 1 inlet and cell 1 pool.

Test organisms survived exposures to water and sediments from the lake site. *C. dilutus* survival was not significantly decreased in sediments collected from wetland sites through 24 hours; however, growth was decreased in sediments from cell 1 inlet and cell 1 pool at 0.5 hours and from cell 2 pool at 24 hours (Table 4). Although decreased survival was measured in sediments collected at 48 hours through 14 days from cell 2 pool, by 26 days *C. dilutus* survival in sediment from this site was not significantly different from controls. Survival was decreased in sediments collected at 26 days from sedimentation pond and cell 1 inlet.

Clam Shell Growth

Clams increased shell size only at sedimentation pond and lake site after 7 days of deployment, whereas shell growth for clams from remaining wetland sites was significantly less than that of control clams ($\alpha = 0.05$) (Table 4). Degrowth continued through 14 days in clams retrieved from cell 2 pool. Although clams from remaining sites had cumulative shell growth, only those from cell 1 inlet had no statistically significant difference in growth from controls. Clams at sedimentation pond, cell 1 inlet, and cell 1 pool did not survive exposures, and shell degrowth was measured for clams remaining at cell 2 inlet and cell 2 pool after 26 days. Clams at lake site produced cumulative shell growth (0.43 ± 0.28 mm) during the time of wetland exposure.

ChE Activity

All clams exposed to diazinon at selected wetland sites had decreased ChE activity except those retrieved after 9 hours from cell 2 pool (Table 4). ChE activity in clams retrieved at 3 hours from sedimentation pool was decreased to 17.5% of control activity (clams deployed in lake site). Although enzyme activity in clams from this site remained low through 14 days, none survived the 26 days exposure. Enzyme activity in clams retrieved at 9 hours from the upstream wetland cell (cell 1 inlet and cell 1 pool) was decreased to 26.9% and 33.4% of controls, respectively, and was always decreased below 25.9% of control activity throughout the remaining exposure. No surviving organisms were retrieved from cell 1 inlet at 26 days, and there was evidence of predation as the cause of mortality for this particular site. ChE activity in clams retrieved at 9 hours from cell 2 inlet was decreased to 25.8% of controls and remained low throughout the exposure. ChE activity measured after 9 hours in clams from cell 2 pool was 75.1% of controls, with enzyme activities decreased below 33.0% of control activity in subsequent samplings.

Discussion

In this study, toxic effects of diazinon movement throughout the wetlands were most apparent in measured *C. dubia* survival (Table 3). Within 0.5 hours after exposure, diazinon had progressed throughout the first wetland cell, resulting in decreased survival through cell 1 pool. Three hours after exposure, *C. dubia* survival was also significantly decreased in water from cell 2 inlet and was transported through this wetland cell, decreasing survival in water from all wetland sites after 9 hours. Measured diazinon concentrations within the wetland remained sufficient to cause *C. dubia* mortality throughout the 24 hours of sampling regime with peak diazinon concentrations at 3 and 24 hours for the first and second wetland cell, respectively. Measured concentrations in the water column after 48 hours indicate a 99% efficiency of the wetlands for diazinon removal. Aqueous samples from all wetland sites with diazinon concentrations lower than acute toxic threshold values (*C. dubia* LC_{50} = 0.47 μg/L) (Bailey *et al*. 1996) after 48 hours and 7 days had significant *C. dubia*

Table 3. Aqueous diazinon concentration in μg/L and mean percent survival of *C. dubia* in laboratory exposures from constructed wetlands after application in 2003

Time after application		Sedimentation pond	Cell 1 inlet	Cell 1 pool	Cell 2 inlet	Cell 2 pool
0 h	aqueous diazinon	ND	NS	0.05	NS	0.08
	C. dubia survival	100	100	100	95	100
0.5 h	aqueous diazinon	102.45	91.69	104.57	ND	ND
	C. dubia survival	0*	0*	20*	65	NS
3 h	aqueous diazinon	95.41	106.63	121.71	182.16	0.07
	C. dubia survival	0*	0*	0*	5*	100
9 h	aqueous diazinon	14.85	14.07	0.06	ND	19.26
	C. dubia survival	0*	0*	0*	0*	0*
24 h	aqueous diazinon	11.46	21.14	47.88	0.02	45.96
	C. dubia survival	0*	0*	25*	0*	5*
48 h	aqueous diazinon	0.18	ND	1.41	ND	ND
	C. dubia survival	0*	0*	15*	10*	0*
7 d	aqueous diazinon	1.10	0.08	ND	0.05	ND
	C. dubia survival	15*	5*	5*	0*	0*
14 d	aqueous diazinon	0.16	1.70	0.43	4.80	0.19
	C. dubia survival	100	100	100	30*	5*
26 d	aqueous diazinon	ND	ND	ND	0.06	0.06
	C. dubia survival	100	30*	0*	0*	0*

NS = not sampled for GC analysis; ND = not detected (lower than LOD). LOD for diazinon in water = 0.01 μg/L.
* Denotes significant difference from controls.

toxicity, indicating the presence of other aqueous toxins, such as pesticide metabolites or breakdown products, remaining in the wetland.

Responses from deployed *C. fluminea* within the wetland cells integrated ambient conditions resulting from the physical, chemical, and biologic attributes of their habitat. With the exception of higher primary production measured in the physicochemical parameters as chlorophyll *a*, comparable conditions existed throughout the wetland sampling sites. In addition to decreased growth and survival of clams from wetland cells, decreased ChE activities resulted from diazinon exposure to deployed organisms. The decreased enzyme activities in clam tissue from upstream sites through cell 2 inlet at 9 hours, demonstrated pesticide transport through the wetland and likely irreversible phosphorylation of ChE as described by O'Brien (1976). Although *C. dubia* failed to survive exposure to water from sampled wetland sites after 9 hours, clams exposed at cell 2 pool had measured ChE activity of 75.1% of controls. This decreased activity would not be considered significant according to Moulton *et al*. (1996) who recommended a 30% decrease relative to control assays as an indication of anti-ChE exposure in freshwater mussels. Although sublethal effects were not measured in deployed clams from this site at 9 hours, aqueous diazinon concentrations (19.26 μg/L) were sufficiently high to cause lethality in *C. dubia*. Marine clams have been shown to display similar delayed enzyme responses (54% of control ChE activity) after an 8-hour exposure to 100 μg/L dichlorvos (LeBris 1995). Valve closure on exposure to aqueous toxicants (Salazar 2003) and decreased algal consumption rates exhibited by clams exposed to metals (Milam & Farris 1998) have been shown to precede such enzyme decreases. However, physiologic effects (Fulton & Key 2001) from ensuing ChE inhibition, and sluggish shell closure (Moulton *et al*. 1996) can also delay or even prevent avoidance of exposure by deployed clams. Because behaviors such as shell closure and escape tendencies (Schulz & Liess 1999) decrease initial toxicant bioavailability (Salazar 2003), these observations should be carefully considered when integrating laboratory biomonitoring and on-site deployment.

In the current study, the toxic effects of diazinon movement in the wetland, as apparent in laboratory biomonitoring with *C. dubia*, elucidated a delayed response of deployed clams most likely caused by avoidance-mediated behavior. Although initial response of *C. dubia* toxicity indicated a potential effect, sublethal effects were evident in later water sampling, and decreased ChE activity in deployed clams indicated a residual toxicant present throughout the study period. This response was also measured in decreased survival of *C. dubia* in samples from downstream wetland sites throughout the 26-day sampling period. Although no toxicity was measured in the most upstream site after 14 and 26 days, clams deployed at these sites survived only through 14 days, with significant decreases in ChE activity.

Resolution of contaminant transport and fate should take into consideration spatial and temporal variation within aquatic systems. Heterogeneity of a wetland system affecting toxicant movement with time, as well as metabolic degradation products resultant from these interactions, increases the complexity of BMP assessment. Common measurements of contaminant fate include partitioning from the water column into sediments, macrophytes, and particulate and dissolved organic material. Although these studies (Ronday *et al*. 1997; Runes *et al*. 2001; Bouldin *et al*. 2004b) provide practical suggestions for BMPs to manage runoff and address water-quality criteria concerns, the array of likely pesticide degradation products is ill defined and costly to measure.

Because better comprehension of such assessments may be gained with appropriate reference conditions (Fulton & Key 2001), enzyme activity, shell growth, and survival of clams placed at lake site were used to characterize conditions for comparable environments lacking pesticide exposure. Adequate conditions to support clam growth were indicated by

Table 4. Measured responses in laboratory and deployed organisms from control and selected wetland sites before and after pesticide exposure.* *Laboratory responses reported as % survival (±1 SD) for 48 h *C. dubia* and % survival and growth as mg (±1 SD) for 10d *C. dilutus* exposures after sample collection. Responses for deployed organisms include cholinesterase activity as % activity for clams simultaneously sampled from control (lake) site and cumulative shell growth in mm (±1 SD) measured after pesticide exposure

Location/organism		Time post dosage								
		0 h	0.5 h	3 h	9 h	24 h	48 h	7 d	14 d	26 d
Lake site (control)										
Laboratory test organisms	*C. dubia* survival	100 ± 0	NS	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
	C. dilutus survival	90 ± 18.5	NS	NS	NS	NS	NS	100 ± 0	100 ± 0	80 ± 27.3
	C. dilutus growth	1.01 ± 0.29	NS	NS	NS	NS	NS	1.55 ± 0.64	2.74 ± 0.32	1.67 ± 0.88
Deployed organisms	Clams retreived (*n*)	NS	NS	NS	12	12	12	12	12	4
	Cholinesterase activity	NS	NS	100	100	100	100	100	100	100
	Shell growth	NS	NS	NS	NS	NS	NS	0.04 ± 0.08	0.2 ± 0.14	0.43 ± 0.28
Sedimentation pond										
Laboratory test organisms	*C. dubia* survival	100 ± 0	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*	15 ± 44	100 ± 0	100 ± 0
	C. dilutus survival	86.7 ± 12.6	75 ± 42.8	85 ± 207	100 ± 0	97.5 ± 5.8	100 ± 0	100 ± 0	86.1 ± 12.6	5 ± 32*
	C. dilutus growth	2.13 ± 0.42	1.48 ± 0.47	1.15 ± 0.43	1.21 ± 0.26	1.30 ± 0.28	1.13 ± 0.37*	1.86 ± 0.68	1.26 ± 0.52	3.16*
Deployed organisms	Clams retreived (*n*)	NS	NS	12	12	12	12	12	12	0
	Cholinesterase activity	NS	NS	17.5*	21.8*	19.1*	15.9*	18.5*	25.3*	NS
	Shell growth	NS	NS	NS	NS	NS	NS	0.01 ± 0.10	0.06 ± 0.09*	NS
Cell 1 inlet										
Laboratory test organisms	*C. dubia* survival	100 ± 0	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*	5 ± 2.1	100 ± 0	30 ± 22.6*
	C. dilutus survival	97.5 ± 5.8	100 ± 0	100 ± 0	NS	90 ± 0.0	100 ± 0	80 ± 21.1	95 ± 6.7	12.5 ± 12.4*
	C. dilutus growth	1.07 ± 0.32	0.77 ± 0.15*	1.79 ± 0.19	NS	1.27 ± 0.51	1.89 ± 0.56	1.91 ± 0.59	2.39 ± 0.66	2.65*
Deployed organisms	Clams retreived (*n*)	NS	NS	NS	12	12	12	12	12	0
	Cholinesterase activity	NS	NS	NS	26.9*	25.9*	14.2*	9.6*	18.0*	NS
	Shell growth	NS	NS	NS	NS	NS	NS	−0.01 ±0.05*	0.16 ± 0.10*	NS
Cell 1 pool										
Laboratory test organisms	*C. dubia* survival	100 ± 0	20 ± 8.2	0 ± 0*	0 ± 0*	25 ± 16.5*	15 ± 4.4*	5 ± 2.1*	100 ± 0	0 ± 0*
	C. dilutus survival	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	95 ± 10.8	100 ± 0	77.5 ± 19.3	100 ± 0
	C. dilutus growth	1.83 ± 0.47	0.82 ± 0.29*	1.87 ± 0.30	2.08 ± 0.18	1.16 ± 0.26	1.99 ± 0.47	2.75 ± 0.36	2.75 ± 0.46	2.65 ± 0.59
Deployed organisms	Clams retreived (*n*)	NS	NS	NS	12	12	12	12	12	1
	Cholinesterase activity	NS	NS	NS	33.4*	25.5*	17.4*	12.8*	16.9*	5.7*
	Shell growth	NS	NS	NS	NS	NS	NS	−0.02 ± 0.03*	0.01 ± 0.08*	−0.09*
Cell 2 inlet										
Laboratory test organisms	*C. dubia* survival	95 ± 8.8	65 ± 33.9	5 ± 2.1	0 ± 0*	0 ± 0*	10 ± 4.0*	0 ± 0*	30 ± 28.3*	0 ± 0*
	C. dilutus survival	100 ± 0	77.5 ± 16.0	95 ± 10.8	95 ± 10.8	80 ± 32.3	97.5 ± 5.8	100 ± 0	100 ± 0	90 ± 18.5
	C. dilutus growth	1.32 ± 0.28	1.71 ± 0.63	1.25 ± 0.30	1.67 ± 0.33	0.75 ± 0.09*	1.66 ± 0.34	1.63 ± 0.69	1.58 ± 0.53	2.15 ± 0.46
Deployed organisms	Clams retreived (*n*)	NS	NS	NS	12	12	12	12	12	4
	Cholinesterase activity	NS	NS	NS	25.8*	10.7*	18.5*	9.1*	19.5*	19.8*
	Shell growth	NS	NS	NS	NS	NS	NS	−0.06 ± 0.08*	0.04 ± 0.06*	−0.09 ± 0.10*
Cell 2 pool										
Laboratory test organisms	*C. dubia* survival	95 ± 8.8	NS	100 ± 0	0 ± 0*	5 ± 2.1*	0 ± 0*	0 ± 0*	5 ± 2.1*	0 ± 0*
	C. dilutus survival	75 ± 42.8	NS	95 ± 10.8	95 ± 6.7	90 ± 18.5	52.5 ± 13.4*	27.5 ± 14.0*	20 ± 11.0*	97.5 ± 5.8
	C. dilutus growth	1.78 ± 0.10	NS	1.89 ± 0.30	1.84 ± 0.29	0.83 ± 0.27*	2.38 ± 0.70	1.86 ± 0.87	1.95 ± 0.54	2.32 ± 0.46
Deployed organisms	Clams retreived (*n*)	NS	NS	NS	12	12	12	12	12	9
	Cholinesterase activity	NS	NS	NS	75.1	33.0*	11.1*	12.1*	17.7*	10.3*
	Shell growth	NS	NS	NS	NS	NS	NS	−0.01 ± 0.05*	−0.01 ± 0.10*	−0.01 ± 0.07*

NS = not sampled.

* Denotes significant difference from controls [a]single value prohibits SD calculation.

comparable chlorophyll *a* values (Foe & Knight 1986) and optimum temperatures (24°C to 30°C) (Doherty *et al*. 1990) measured from cell 2 pool and lake site. Although shell degrowth was measured in clams from wetland sites, shell growth was measured in clams from lake site and enzyme activity from these control organisms enabled a calculation of percent control ChE activity for all other affected sites.

Although diazinon concentrations dissipated from the water column throughout the 26-day exposure, toxicant bioavailability to deployed clams continued. During this study, filter-feeding from the water column, combined with pedal-feeding of sediment organic matter by *C. fluminea* (Way *et al*. 1990), seemed to have contributed to clam exposure to diazinon during partitioning to plant and sediment organic matter. Pesticide or metabolites bound to sediments contributed to the decreases in growth and ChE activities in deployed clams. Because the Asian clam has been proposed as an important coupler between benthic and pelagic processes (Hakenkamp & Palmer 1999) and also used in deployments to decrease uncertainties of traditional laboratory bioassays (Salazar & Salazar 1997), responses from these organisms should provide significant advantages in assessment of agricultural runoff. Although effects associated with decomposition and particulate organic matter would be reflected in *C. fluminea* responses, decreased survival of *C. dilutus* exposed to sediment collected at 26 days may have also reflected a bioavailable toxicant and subsequent route of exposure caused by the ingestion of sediment collected from upstream wetland sites.

Spatial heterogeneity within vegetated systems and described bioavailability with vegetative interaction may have been observed in this study regarding *C. dubia* survival in water with diazinon concentrations higher than toxic thresholds. Conversely, the presence of pesticide metabolites most likely resulted in decreased *C. dubia* survival in samples with no detectable diazinon. The importance of bioassays in the absence of measured pesticide metabolites during remediation was emphasized by Belden *et al*. (2004), who observed discriminations in toxicity with measured pesticide concentrations. Decreased ChE activity, survival, and growth of deployed clams throughout the study also reflected prolonged interaction with a bioavailable toxicant within the wetland system. Site conditions experienced by deployed organisms incorporating benthic and aqueous exposure routes better quantified available toxicants through integration of pesticide partitioning and metabolites. Supplementation of filter feeding by deposit feeding, especially in organic rich substrates (Way *et al*. 1990), is expected to increase the bioavailability of pesticides and their metabolites throughout their transformation within a wetland. Deployed organisms offered an evaluation of techniques for quantifying toxic effects in agricultural mitigation areas to incorporate site-specific interactions.

Combined assessments of laboratory biomonitoring and deployed organisms in this field investigation provided a risk-management evaluation tool of environmental effects of diazinon within a recognized agricultural BMP. Vertical wetland cells in this study served as an ecotone by separating agricultural runoff from its receiving stream. The spatial separation of this BMP provided time for diazinon to dissipate from the water column providing protection to downstream communities. Although metabolites were not measured in this study and significant concentrations of diazinon dissipated from the water column within 48 hours, possible effects from pesticide byproducts may have had lingering effects to test organisms. Use of these techniques proximal to receiving streams may provide a comprehensive assessment of the interactions within the components of the agricultural landscape and provide a risk-management tool to assess effectiveness of upstream agricultural BMPs.

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