Effectiveness of Agricultural Management Practices in Reducing Concentrations of Pesticides Associated with Toxicity to Aquatic Organisms

Data Summary and Final Report

Submitted to

THE RESOURCE CONSERVATION DISTRICT OF MONTEREY COUNTY,

THE CENTRAL COAST REGIONAL WATER QUALITY CONTROL BOARD, AND

THE CALIFORNIA STATE WATER RESOURCES CONTROL BOARD

Submitted by

THE MARINE POLLUTION STUDIES LABORATORY

UNIVERSITY OF CALIFORNIA AT DAVIS,

DEPARTMENT OF ENVIRONMENTAL TOXICOLOGY

March 30, 2007

Authors:

John Hunt[1], Brian Anderson[1], Bryn Phillips[1], Bryan Largay[2], Fred Watson[3], Kelleen Harris[3], Emily Hanson[4], Melanie Beretti[4], Ruthie Schafer[4], Karminder Brown[4], and Amanda Bern[5]

[1]Department of Environmental Toxicology, University of California at Davis

[2]Largay Hydrologic Sciences, LLC

[3]Central Coast Watershed Studies, California State University, Monterey Bay

[4]Resources Conservation District of Monterey County

[5]Central Coast Regional Water Quality Control Board

(Please note that a CCoWS CSUMB report [Harris et al., 2007, in prep.] presents design and flow details, as well as complimentary monitoring data from the Tembladero Slough constructed wetland.)

This report may be cited as:

Hunt JW, Anderson BS, Phillips BM, Largay B, Watson F, Harris K, Hanson E, Beretti M, Schafer R, Brown K, Bern AL. 2007. Effectiveness of agricultural management practices in reducing concentrations of pesticides associated with toxicity to aquatic organisms. Data Summary and Final Report. Central Coast Regional Water Quality Control Board, San Luis Obispo, CA.

Acknowledgements

We thank Ross Clark of the California Coastal Commission for facilitating partner support in the construction and operation of the vegetated treatment system in the Tembladero Slough constructed wetland. Those partners included the Moss Landing Marine Laboratories, Central Coast Watershed Studies (CCoWS) at the California State University Monterey Bay, the Resource Conservation District of Monterey County, and the Community Alliance with Family Farmers. The Monterey County Water Resources Agency assisted with site development. Adam Wiskind, Rob Burton, Gage Dayton, and Cara Clark from MLML, and Fred Watson from CSUMB were instrumental in creating the constructed wetland. Adam Wiskind deserves particular mention for planning, securing, and operating wetland facilities during the course of the study.

Sara Clark, Jason Flynn, Witold Piekarski, Katie Siegler, and Jennifer Voorhees of the Marine Pollution Studies Laboratory conducted toxicity tests, field work, and data management; and contributed in many fundamental ways to the completion of this project.

Kelleen Harris led the CCoWS CSUMB field and laboratory team that included: Miles Daniels, Peter Martin, Brad Travers, Marc Los Huertos, Regina Williams, Morgan Wilkinson, Doug Smith, Jessica Watson, and Shana Carmichael. Miles Daniels calculated and controlled flow rates in the wetland during the monitoring studies.

Dave Crane, Abdu Mekebre, Mary Curry, and Loc Nguyen of the California Department of Fish and Game Water Pollution Control Laboratory conducted high quality organic chemical analyses and compiled extensive data reports and quality control documentation.

The Resource Conservation District of Monterey County was instrumental in working with cooperating growers to design and construct on-farm vegetated treatment systems.

We are grateful to the cooperating growers for their time, expertise, commitment of land and resources, and dedication to improving water quality along the Central Coast.

Laura Lee Leink, Christina Hurley-McKnew, Peter Nelson, Julie Dustin, Bree Candiloro, Emily Smith, and the Greenthumbs of Return of the Natives, and Kellie Rey and Cara Clark of Coastal Conservation and Research were instrumental in establishing vegetation in the Tembladero Slough constructed wetland.

Executive Summary

The California central coast is one of the most productive agricultural areas in the world. Soils, weather, and an innovative industry combine to produce most of the nation's salad greens, as well as many other vegetables and specialty crops. Production techniques have included use of synthetic nutrients and pesticides, and transport of these compounds in agricultural runoff has been associated with adverse effects on aquatic ecosystems.

Numerous stakeholders have worked to implement conservation practices in agricultural operations to reduce the impacts of contaminated runoff. These practices include construction of vegetated treatment systems (VTS) designed to promote contaminant reduction and breakdown. Different types of contaminants are mitigated by different treatment processes. For example, nitrate is reduced primarily through denitrification, which occurs when water remains in extended contact with soil-associated anaerobic micro-organisms. Pesticide reductions occur via hydrolysis, through extended solution in water; photolysis, through exposure to sunlight; sedimentation, through particle binding and settlement; retention on plant surfaces and associated breakdown by attached micro-organisms; and dilution of chemical pulses in retention ponds and channels.

As continuing effort is applied to implementing management practices, practice evaluation studies are necessary to demonstrate their effectiveness and to test system modifications for optimizing contaminant reduction. This study was designed to evaluate the effectiveness of two types of VTS systems: a constructed wetland, and VTS ponds constructed by cooperators on working agricultural operations. The study was designed to collect water and sediment samples at VTS inlets and at various stations within the systems. These samples were tested for toxicity to aquatic invertebrates. Toxicity identification evaluations (TIEs) were conducted to identify chemicals responsible for observed toxicity, so that VTS evaluations could focus on the chemicals of greatest concern. Samples were analyzed chemically to determine concentrations of pesticides and nutrients, and the trends in these concentrations were analyzed relative to location in the VTS. The wetland system was evaluated to compare pesticide reductions with water flow rate. Focused studies were conducted to track a parcel of pesticide contaminated water through the constructed wetland,

and to evaluate the relationship of hydrologic factors to changes in pesticide concentrations in the VTS pond systems.

There were a number of constraints on study design and outcome. For much of the study duration, insufficient information was available about pulse inflows and VTS hydrology to predict when an influent parcel of water would arrive at the VTS outflow or other points in the system. Thus, water measured at the inlet was not necessarily the same water measured downstream. To address this concern, inlets at the VTS ponds were sampled on three consecutive days to create a composite sample against which to compare samples from other points in the system. At the constructed wetland site, dissolved salt concentrations exceeded expectations in the late summer and fall surveys, so the toxicity test organism, *Ceriodaphnia dubia*, had to be replaced with the more euryhaline amphipod *Hyalella azteca*, which is also less sensitive to some of the pesticides of concern in that system. A final caveat is that this study was not designed to elucidate the mechanisms of observed pesticide reduction. Some analysis of relationships between suspended particles and pesticide concentrations were conducted, but the information gathered here was not intended to allow a thorough investigation of the contributions made by various mechanisms, including hydrolysis, photolysis, plant surface sorption, microbial breakdown, particle settling, and dilution.

A large number of measurements were made during this study (Appendix A, pages 1 to 173). All planned measurements were completed. One planned TIE was replaced by an intensive characterization of diazinon behavior in relation to VTS hydrology. Data quality for the study was very high, as documented in the Quality Assurance section of the Methods chapter.

A number of interesting results were observed. Water samples from the Tembladero Slough (which was pumped into the constructed wetland VTS) were toxic to *C. dubia*. This toxicity appeared related to total organophosphate pesticide concentrations. In early surveys using *C. dubia*, toxicity and pesticide concentrations decreased with distance traveled through the constructed wetland. Pesticide reductions tended to increase with residence time in the system. Organochlorine pesticide concentrations for were related to suspended particle concentrations. Pesticide reduction with distance from the inlet was not observed in all surveys; but when a diazinon-laden parcel of water was tracked through the system over its three-day residence time, diazinon concentrations decreased by dilution, as did toxicity.

In the on-farm VTS ponds, nearly all inflow samples were highly toxic to *C. dubia*. TIEs and subsequent chemical analyses indicated that chlorpyrifos was responsible for toxicity in water and sediment samples from site G-09, and that the pyrethroid pesticide permethrin also contributed to sediment toxicity. High influent concentrations of chlorpyrifos at G-09 decreased to much lower concentrations at the VTS outlet in all five surveys. Concentrations of most pesticide classes were lower at the G-09 outlet than at the inlet, indicating overall VTS effectiveness in reducing pesticide concentrations.

At the SV-03 VTS site, influent samples were toxic to *C. dubia* in four of five surveys. TIEs indicated that toxicity was due to diazinon, which occurred at concentrations as high as 9.6 ug/L. There was strong TIE evidence that sediment sample toxicity at this site was due to high concentrations of the pyrethroid pesticides lambda-cyhalothrin and cypermethrin. Diazinon concentrations generally did not decrease in the VTS, though the high (9.6 ug/L) diazinon inflow pulse was not seen at the system outlet. Concentrations of most pesticide classes were lower at the SV-03 outlet than at the inlet, again indicating overall VTS effectiveness in reducing pesticide concentrations. A focused study of VTS hydrology and diazinon concentration at SV-03 indicated that complex in-pond dilution processes were responsible for dampening pulse inflow peak concentrations, so that outflows had generally lower concentrations.

The overall conclusion that can be drawn from these analyses is that these VTS systems were effective at markedly reducing pesticide and nitrate concentrations. Most pesticides showed declines in most cases. Water soluble pesticides such as diazinon may not be broken down during the VTS residence times observed in this study; but focused studies, in which parcels were carefully tracked and hydrology was better characterized, showed notable diazinon reductions via dilution of peak concentrations within the systems.

Further work should include thorough initial hydrologic characterizations to allow more precise measurements of treatment effectiveness, as well as mass balance investigations to determine which components of the systems are responsible for pesticide and nutrient reductions. These studies will greatly increase our knowledge of system properties, and allow the design of increasingly efficient conservation practices to mitigate the effects of non-point source runoff to the critical aquatic habitats of the central coast.

Table of Contents

List of Tables

List of Figures

List of Figures

(continued)

INTRODUCTION

The coastal valleys of central California are considered the nation's salad bowl, forming the heart of the most productive vegetable producing region in the country (California Farm Bureau; http://www.cfbf.com, 2005). This region contains year-round, intensively cultivated agricultural land supporting a nearly $5 billion/year industry producing most of the nation's salad greens, artichokes, and crucifer crops. Runoff from irrigated agriculture constitutes a significant portion of stream flow in the region during most of the year, and a number of studies have documented pesticide occurrence and biological impacts in the rivers and estuaries of the Pajaro (e.g., Hunt et al. 1999), Salinas (e.g., Anderson et al. 2003) and Santa Maria watersheds (e.g., Anderson et al. 2006). In the lower Salinas and adjacent Gabilan watersheds, thirteen water bodies are currently listed as impaired by pesticides and/or nutrients under Clean Water Act §303[d], including Gabilan, Natividad and Alisal Creeks, the lower Salinas River, and Tembladero Slough.

Evidence of water quality impact has motivated a number of diverse stakeholders to begin implementing farm management practices (MPs) to reduce nutrients, pesticides, and toxicity in agricultural runoff. Farm owners and managers have worked with the Farm Bureau, the Agricultural Commissioners, the Natural Resources Conservation Service (NRCS), the Resource Conservation Districts (RCDs), UC Cooperative Extension (UCCE), and others to design and construct vegetated treatment systems in ponds, buffer strips, and ditch channels at a variety of locations throughout the region. Successful MP development and water quality improvement now depend on continuing evaluation and modification of these practices to determine and optimize their effectiveness in reducing the loadings of non-point source pollutants.

The current study was designed to evaluate the effectiveness of farm management practices and stream restoration efforts in reducing contaminants in agricultural runoff. The study focused on two types of projects:

1. vegetated ponds constructed on farm property to hold agricultural runoff and allow contaminant reduction to occur through a process of retention, sorption and breakdown; and

2. a constructed wetland channel and marsh, designed to treat contaminants in slough water draining from a large agricultural watershed, so that loadings are reduced before runoff enters critical wildlife habitat.

Study approach

The on-farm treatment pond systems in the Gabilan watershed were constructed by interested growers in the context of their field production activities, with design assistance from the RCD of Monterey County (RCDMC) and others. Measurements of chemistry, toxicity, and ancillary parameters in water and sediment were conducted by scientists from the UC Davis Marine Pollution Studies Laboratory (MPSL), with assistance from the Monterey RCD. Measurements related to water flow and retention timing were made by Largay Hydrologic Sciences, LLC. The Tembladero Slough constructed wetland (Figure 1) was located on land owned by a local agency, and was constructed by contractors under the direction of scientists from the Moss Landing Marine Laboratories (MLML) and California State University, Monterey Bay (CSUMB). Results of hydrologic, nutrient, and other evaluations of this system are presented in a separate report produced by the CSUMB Central Coast Watershed Studies (CCoWS) group (forthcoming).

The basic sampling design for the on-farm vegetated treatment systems was to collect samples of water flowing into the pond, and of water flowing out of the pond at the downstream end. One system, at site SV-03, consisted of two ponds in series, so a third sampling station was added here to collect water just before it flowed out of the first pond and into the second. Because of insufficient information regarding pond residence times, composite samples, consisting of grab samples collected on three successive days, were used to characterize water at the inflow. Water samples were collected in five separate surveys over a four month period during the summer/fall 2006 irrigation season. Pond sediments were sampled once. Details of the samples and analyses are given below. The objective of the pond studies was to investigate change in pesticide concentrations and toxicity from inflow to outflow, as a way of evaluating system effectiveness.

The sampling design for the Tembladero Slough constructed wetland is given below. The approach here was to conduct eight separate surveys, each coinciding with a different pumping

rate and treatment channel residence time. MPSL staff collected samples for pesticide chemistry and toxicity testing, and CCoWS staff collected samples for suspended sediments, nutrients, and conventional parameters. Trays were submerged in the channel to collect sediment particles settling out of the water column, and these solid-phase samples were used for chemical and toxicological analyses. The objectives of the Tembladero wetland study were to measure water and sediment characteristics at different points in the system, to examine system effectiveness in improving water quality, to evaluate relationships between parameters to better understand treatment processes, and to investigate the relationship between pumping rate and water quality improvement.

Each system was evaluated by considering the different types of analyses in sequence. Toxicity data were examined first. These data were used to determine whether contaminants were present in biologically relevant concentrations, and to select subsets of samples for toxicity identification evaluation (TIE). In the vegetated treatment pond studies, TIEs were conducted to determine which chemical(s) were implicated as the cause(s) of toxicity, and this information was used to focus system effectiveness evaluations on the chemicals of greatest concern. Toxicity data were then matched with chemistry data to look for trends in contaminant reduction in the vegetated treatment systems. Finally, pesticide concentrations and their reduction were considered relative to factors affecting their removal from the water column, such as flow rate, sedimentation, retention, and breakdown.

Caveats

A number of factors complicated evaluations of treatment system effectiveness, and these should be kept in mind when considering study results.

The treatment pond systems were constructed as part of working farming operations, and were affected by pre-existing field and drainage conditions. The configurations of both pond systems changed in significant ways over the course of the study. At the beginning of the evaluation process, both ponds had multiple inlets, including direct field runoff entering at various points. At site G-09, there was a main inlet at the upstream head of the pond, with three additional inlets along one side (Figure 2) which drained seven percent of the watershed. In the two-pond system at SV-03 (Figure 3), there was a secondary inlet located in the downstream pond near the system outlet, which drained eight percent of the watershed. Field

runoff from this secondary inlet bypassed the upper treatment pond entirely and may have channeled through the second pond with minimal residence time. This inlet was removed after the second survey, but may have affected subsequent results, as well.

Hydrologic characterization of these pond systems occurred concurrently with water quality assessment during the short time window available for this study. Inflows were inconsistent and depended on production irrigation schedules that were managed independently, such that water was delivered to the ponds in pulses of varying duration and volume. Predicting residence time under these conditions is complex. Because of these circumstances, the water quality surveys were conducted in the absence of adequate information for estimating pond residence times. Without this information, we could not track pulses of water flowing through the ponds, and instead relied on a 3-day composite at the inflow, collecting the outflow samples on the third day. It appeared at the end of the study that residence times were longer than one to three days, and that water sampled at the outlets was very likely not the same water sampled at the inlets. Direct comparisons of pesticide or nutrient concentrations between pond inflows and outflows would depend on the assumption of constant contaminant concentration in inflowing runoff. There is very little reason to suspect that inflow concentrations were constant, and past experience indicates that runoff concentrations are highly variable. Despite this complication, some trends were apparent in the data collected, but the reader should keep this situation in mind when considering the study results.

The same situation existed to some extent with surveys of the Tembladero constructed wetland. While the channel morphology and water pumping rates were known throughout the study, complications due to sample holding time requirements and flow variations led to design decisions to sample all stations within the channel and wetland on the same day, rather than attempt to track a pulse of water through the system.

In addition to this caveat, other system features must be kept in mind. The Tembladero Slough, from which water was pumped into this treatment system, is slow moving during the summer, and empties into a brackish estuary. Tide gates downstream limit the intrusion of salty surface water up the channel, but the Slough did become stratified during the study period, with higher conductivity water at the bottom. The 3-inch diameter hose that drew water from the Slough was placed at the Slough bottom. Water samples collected from the

surface of the Slough showed that surface waters were less saline and had higher pesticide concentrations than bottom water drawn into the system by the pump. Once these data were available, a float was attached to the pump hose in December 2006, so that surface water was pumped into the system during the final assessment of stormwater treatment. Because Tembladero Slough bottom water was pumped during the majority of sampling events, the conductivity of Slough water increased during the study period, so that by August 2006, conductivity was too high for toxicity testing with the sensitive species *Ceriodaphnia dubia*. This necessitated switching to a more euryhaline test species, the amphipod *Hyalella azteca*. This species is less sensitive to organophosphate pesticides, which are pesticides of concern in this system, so some ability to detect the presence and toxicity of bioavailable chemicals was compromised.

While conductivity was measured during each survey, two sets of toxicity tests were conducted using water in which conductivities were too high for the test organism used (*Ceriodaphnia dubia*). The test organism was replaced with *H. azteca*, and all subsequent tests were doubled, so that each used *H. azteca* on full strength samples and *C. dubia* on dilutions with adequately low conductivity (but concomitantly lower pesticide concentrations). Toxicity tests from the two surveys that were confounded by high conductivity could not be repeated, because the overall study design depended on changing flow rates at specified intervals. (Details of system design are available below and in the companion CCoWS report.)

System Effectiveness and Environmental Fate of Pesticides

In order to design and conduct a study of management practice effectiveness, some properties of pesticides must be considered in relation to the components of the treatment systems being evaluated. It is also important to note that because this study made extensive use of invertebrate toxicity testing to detect compounds present in concentrations of concern, insecticides tended to receive more attention here than herbicides or other constituents that influence aquatic communities through different mechanisms.

Vegetated treatment systems reduce chemical contaminants via several mechanisms. These include removal through sorption of chemicals to plant surfaces, removal of particle-bound contaminants through sedimentation, and reduction via chemical and biological degradation.

The relative roles of these mechanisms are determined in part by the chemical characteristics of the different classes of pesticides, including their solubility, their volatility, and their susceptibility to bacterial, photolytic, and hydrolytic degradation under different physical and chemical conditions. These characteristics influence the efficacy of vegetated treatment systems in removing contaminants and determine the most appropriate system components for each class of chemical.

Pyrethroids

Pyrethroid pesticides are degraded by both biological and chemical processes (hydrolysis and photolysis), with chemical degradation playing a larger role in the environment. However, the synthetic pyrethroids have been engineered to be fairly stable to hydrolytic and photolytic degradation (Coats et al. 1989). Their hydrolysis half-life in aquatic environments is typically on the order of weeks to months at lower pH (e.g., pH 7), and days to weeks at higher pH (e.g., pH 9). Their photolysis half-lives in water are generally on the order of weeks to months, and the aerobic half-life in soils is on average 30-100 days for most pyrethroids (Hendley et al., 2006). These data suggest that within the time frame of residence times expected in typical vegetated treatment systems, there will be insufficient retention time to allow significant chemical degradation of pyrethroids in water. Because of their hydrophobicity (log Kow = 5.5 – 6.0), removal of pyrethroids via sorption to plant surfaces and deposition of particle bound pyrethroids in treatment system sediments will play a much larger role in reducing the off-site transport of these pesticides (e.g., Moore et al. 2001, Schultz 2004).

Organochlorines

Many legacy organochlorine pesticides (e.g., dieldrin, DDT, chlordane) are relatively stable in the environment, having half-lives in soils of many years. Biotransformation by natural bacterial populations is the most significant pathway for metabolism in sediments, but is too slow a process to be meaningful in vegetated treatment systems. Most organochlorine pesticides are relatively hydrophobic, and are therefore bound to particles and organic matter (e.g., DDT and DDT metabolites log Kows = 6.10 – 6.76). Therefore, as with the pyrethroid pesticides, treatment systems that enhance contact with plant surface area and promote removal of particles via sedimentation will be more effective at removing organochlorine pesticides.

Organophosphates

Diazinon degrades via photolysis and hydrolysis. Photolysis half-lives in water are on the order of days to weeks (Novartis, 1997). Hydrolysis half-lives are on the order of days at low pH (pH = 5), to weeks to months at neutral to higher pH (pH = 7 - 9). Because it is more water soluble (log Kow = 3.7), removal of diazinon via its sorption to plant surfaces and deposition in sediments is less efficient than has been observed with the more hydrophobic pesticides (e.g., Watanabe and Grismer, 2001). Therefore, treatment systems that are designed to increase retention time, in combination with treatment areas that facilitate chemical degradation, will be more effective at removing diazinon. Chemical degradation will be more efficient in treatment systems that allow good UV penetration (for photolysis) and that are characterized by lower than neutral pH to enhance hydrolysis. Chlorpyrifos is also removed from water via hydrolysis (half-life = 16 to 72d at pH 5-9) and photolysis (half-life = 30 to 52d), and via bacterial degradation under anaerobic conditions (Giesey et al., 1999). Hydrolysis is the primary degradation process and hydrolysis rates are enhanced under alkaline conditions (higher pH). Chlorpyrifos (log Kow = 5.26) is much less water soluble that diazinon, and is therefore more likely to be bound to particles in solution. This organophosphate is therefore more amenable to removal via sorption to plant surfaces and in systems that promote particle deposition (e.g. Sherrard et al. 2004).

Carbamates

Like diazinon, the carbamate pesticides are relatively water soluble (e.g., carbaryl log Kow = 2.36, carbofuran log Kow = 2.32). Because of this characteristic, carbamates are less likely to be reduced in vegetated treatment systems via sorption to plant surfaces or through sedimentation. The two primary carbamate degradation pathways are via hydrolysis and photolysis. Hydrolysis half-lives for carbaryl are on the order of hours at pH 9 to days at pH 7. A photolysis half life of 21 days has been reported for carbaryl under artificial light in water, and has been reported to be on the order of a few days under natural lighting conditions (Xu S., 2000). These characteristics suggest that systems that allow sufficient retention time for hydrolysis (under neutral to slightly alkaline conditions), and that promote UV light penetration for photolysis will be more effective at removing carbamate pesticides.

Nitrate is often of concern in surface waters because excess nutrients promote algal growth and eutrophication, which can lead to extreme variations in dissolved oxygen concentration sometimes related to fish kills and other impacts. Vegetated treatment systems optimized for nitrate removal are often designed to maximize water contact with soil, through incorporation of broad, shallow areas. Anaerobic micro-organisms associated with submerged soils can denitrify overlying water, converting nitrate into gaseous nitrogen (N_2), which can escape into the atmosphere.

METHODS

Tembladero Wetland Study Area

The constructed wetland project is adjacent to the confluence of the Tembladero Slough and the Old Salinas River Channel, and is located at the corner of Monterey Dunes Colony Road and Molera Road (Figure 1). Scientists from the Moss Landing Marine Laboratories excavated a channel in bench lands that are elevated just above the high tide line. The restoration project consists of a sinuous vegetated channel designed to adsorb and breakdown pesticides, followed by a wetland spreading area designed to foster water/soil contact to aid in denitrification. Vegetation in this system is dominated by cattails (*Typha sp*.), which were planted on submerged sills built across the channel to promote vertical mixing and vegetation contact. For specified hours each day during the study, water from the Tembladero Slough was pumped into the head of the channel, which was excavated to greater depth to serve as a sedimentation basin. Water then flowed through the vegetated channel and shallow wetland before re-entering the Slough just above the confluence with the Old Salinas River. Water samples were collected near the pump intake in the slough (TEM-MOL), at the input pipe in the upper sediment basin (DCR-001), at approximately halfway through the vegetated channel (DCR-002), at the outlet pipe of the channel (DCR-003), and at the outlet of the wetland (DCR004).

Figure 1. Site diagram of constructed wetland at Tembladero Slough.
Water is pumped from the slough (at station TEM-MOL – near submersible pump intake) and enters the constructed channel at station DCR-001. Station DCR-002 is midway down the channel, and station DCR-003 is the channel outlet, where water flows to the shallow wetland. Station DCR-004 is the outlet from the wetland, where water flows back into the slough.

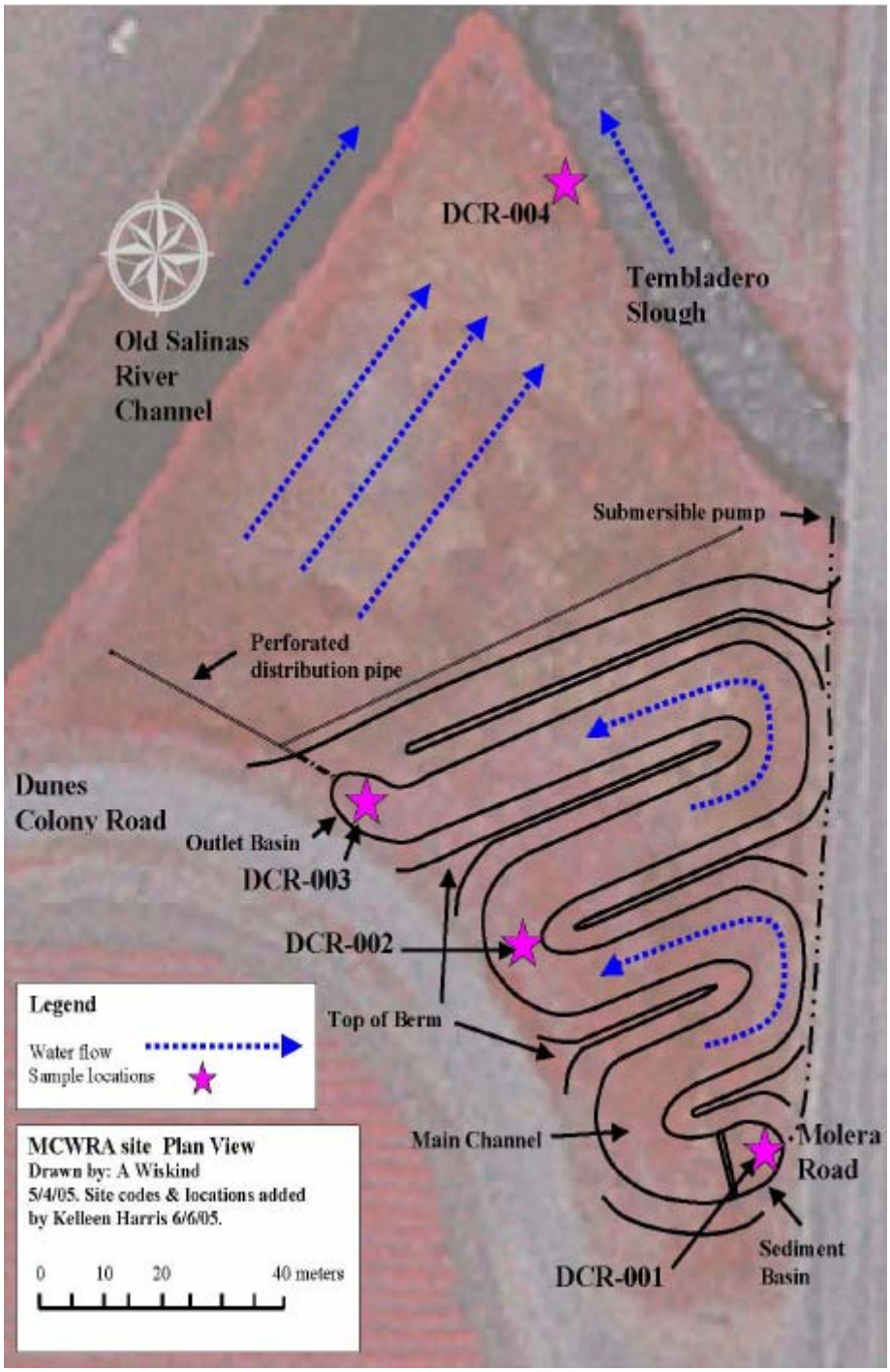


Diagram courtesy of Central Coast Watershed Studies group, California State University Monterey Bay.

On-Farm Vegetated Treatment System Study Areas

The agricultural management practice systems are retention ponds that collect drainage water from a greenhouse flower growing operation (G-09), and a 120-acre field of row crops planted in lettuce, broccoli, and various other vegetables (SV-03).

G-09 is a vegetated treatment system (VTS), approximately 70 m by 12 m, which collects drainage water from 85 acres of greenhouse operations, through one primary input and three secondary inputs (Figure 2). The primary input drains the majority of the greenhouse property, and the secondary inputs drain three two-acre outdoor grow-out areas for flats of newly planted flowers. While the primary input maintains a constant flow, the secondary inputs flow intermittently to the pond. No attempt was made to quantify the relative contributions of the secondary inputs. The primary input approaches the pond through an open ditch

Figure 2. Photograph of vegetated treatment pond at site G-09.

Photograph courtesy of Largay Hydrologic Sciences, LLC

before entering a culvert into the pond. The culvert enters the pond approximately one meter below the surface. Water drains from the pond through a second culvert into an open ditch. Water samples for toxicity and chemistry were collected in the ditch before the input culvert (G-09 A) and in the ditch outside the exit culvert (G-09 B). Sediment samples were collected in the input ditch (G-09 A) and in the pond immediately in front of the exit culvert (G-09 B). Three aquatic plants grew in the G-09 pond: duckweed (*Lemna sp*.), watercress (*Nasturtium sp.*), and pennywort (*Hydrocotyle spp.*). Pennywort was the dominant species in this system.

SV-03 is a two-pond system, with a 27 m x 12 m x 1 m deep upper pond, and 24 m x 12 m x 2 m deep lower pond (Figure 3). The upper pond at SV-03 has a single primary

Figure 3. Photograph of vegetated treatment pond at site SV-03.
View is looking upstream from lower pond outlet toward upper pond, fields, and ditch draining furrow runoff toward the pond.

Photograph courtesy of Largay Hydrologic Sciences, LLC.

input that drained through a flume, but there was also a secondary input to the lower pond that was re-routed into a separate drainage system after the second survey of this study. The primary input drains furrow runoff from approximately 120 acres of irrigated row crops, while the secondary input drained approximately 9 acres. The SV-03 ponds are joined by a single culvert, and the lower pond is drained by a single culvert. Water samples for toxicity and chemistry were collected at the primary input in the flume (SV-03 A), at the exit of the upper pond (SV-03 B), and at the exit of the lower pond (SV-03 C). Sediment samples were collected below the flume at the input (SV-03 A) and at the exit of the lower pond (SV-03 C). Pennywort (*Hydrocotyle spp.*) was the only plant species in this system.

Sample Collection

Water samples were collected in 2.5-liter amber glass bottles. Bottles were rinsed three times with site water before filling. Bottles were filled at least one cm below the surface to avoid floating debris and the surface microlayer. At several stations the bottles could not be filled in this manner. At these stations the bottles were filled from pipes or flumes. Eight sets of water samples were collected from the Tembladero study area and five sets of water samples were collected from each of the farm pond study areas between July and November 2006. Bottles were immediately placed in coolers with sufficient wet ice to adjust and maintain the temperature at 4 ± 3° C during transport to the Marine Pollution Studies Laboratory (MPSL).

To collect sediment in the Tembladero study area, Pyrex trays were placed at stations DCR001, DCR002, and DCR003. Suspended sediment from water pumped into the channel settled into the trays as it passed by the stations. At four and six week intervals the trays were covered, removed from the channel, and emptied into two-liter glass jars for transport to MPSL. There they were homogenized and split for chemical analysis and for toxicity testing with the amphipod *H. azteca*. Sediment samples were collected in the Tembladero study area during three separate surveys. Sediment samples were collected from the farm pond study areas during one survey. Surficial sediments from G-09 were collected using a polycarbonate scoop and sediments from SV-03 were collected using a polycarbonate core tube. Sediments from both sites were placed into 12-liter

polycarbonate tubs for chemical analysis and toxicity testing with *H. azteca*. Each pre-cleaned container had waterproof printed labels attached prior to use in the field. Labels identified site and sampling date.

Water samples were stored at 4 ± 3° C for no longer than 48 hours prior to toxicity test initiation. After a minimum of 16 hours in storage, water samples were decanted to separate overlying sample from settled particulates. Decanted water was poured through a 25-µm pre-cleaned Nitex® screen to remove fauna and larger buoyant particulates. A separate screen was used for each sample. Samples were placed in the constant temperature room at test temperature to acclimate for 24 hours prior to testing. Sediment samples were tested within 14 days.

Toxicity Testing

Water toxicity was evaluated using the 7-day chronic *Ceriodaphnia dubia* toxicity test (U.S. EPA 2002). Each undiluted sample was tested using 10 replicates containing one *C. dubia* neonate (< 24-h-old, obtained from in-house cultures). Survival and reproduction were monitored daily. Water quality parameters including conductivity, hardness, alkalinity, pH, dissolved oxygen, and ammonia were measured at the beginning of each test. Test solutions were renewed daily, and dissolved oxygen and pH were measured on the old solution. Dissolved oxygen was measured on the new solution.

Sediment toxicity was assessed using the 10-day growth and survival toxicity test with *Hyalella azteca*, a resident epibenthic amphipod (US EPA 2000). Each sample was divided among eight laboratory replicates, each with ten 7- to 14-day-old amphipods. The amphipods were exposed to 100 mL of sediment in 300 mL beakers, each containing 175 mL of overlying water. The test temperature was 23 ± 1°C. Water quality parameters, including dissolved oxygen, pH, conductivity, and ammonia, were measured at the beginning of each test. Hardness and alkalinity were measured at the beginning of each test. Overlying water was renewed twice daily, and 1.5 mL YCT food (yeast, cerophyll, and trout chow) was added daily to each test container. The containers were not aerated, but dissolved oxygen was measured daily. After surviving animals were dried at the end of the test, growth was measured as change in mean dry weight per individual amphipod per replicate. *H. azteca* were obtained from Chesapeake Cultures

(Hayes, VA) 48 hours prior to test initiation. The culture was maintained at 23°C and fed YCT (US EPA 2002).

Water quality parameters of dissolved oxygen, pH, and conductivity were measured using a Hach SensION selective ion meter with appropriate electrodes; and ammonia, nitrate, and phosphate were measured using a Hach 2010 spectrophotometer. Temperature was measured using a continuously recording thermograph and thermometer.

Toxicity Identification Evaluations (TIEs)

TIEs were performed on both water and sediment samples. A water column TIE with *C. dubia*, a solid-phase TIE with *H. azteca*, and an interstitial water TIE with *H. azteca* were conducted on water and sediment samples from each input to the retention ponds at the G-09 and SV-3 study sites.

The following solid-phase TIE treatments were performed on undiluted sediment. Treatment blanks consisted of laboratory formulated sediment that underwent the same treatment as the sample. Formulated sediment was prepared using equal parts Salinas River, California, reference site sediment and clean, kiln-dried sand (#60, RMC Pacific Materials, Monterey, CA, USA). The sediment was amended with 0.75% organic peat moss (Uni-Gro, Chino, CA, USA). One kilogram (dry weight) of formulated sediment was prepared by combining 500g reference sediment, 500g sand, and 7.5g peat with 350 mL clean dilution water. Phase I TIE treatments consisted of additions of amendments to the sediment, or treatments of the overlying water. Sediment amendments included Ambersorb (organics) and SIR-300 (metals) binding resins. Overlying water treatments consisted of addition of a carboxylesterase enzyme, bovine serum albumin (BSA), and piperonyl butoxide (PBO). The baseline treatment was also performed at a colder temperature to determine if pyrethroids caused toxicity. Phase II TIE procedures consisted of separating the Ambersorb resin beads from the sediment, extracting them with solvent, and spiking control water with the acetone eluate to determine if toxic concentrations of non-polar organic chemicals could be recovered from the resin.

The following Phase I solid-phase TIE treatments were performed on undiluted solid-phase sediments:

• Baseline – Toxicity test on un-manipulated sample. Five 250-mL replicate beakers each containing approximately 50g sediment and 200 mL clean dilution water.

• Ambersorb 563® (Rohm and Haas, Spring House, PA, USA), a carbonaceous, non-polar resin, was prepared by rinsing it thoroughly with Nanopure® water. Ten percent Ambersorb by wet weight was added to sediment (Kosian et al. 1999, West et al. 2001). Treated sediment was homogenized for 24 hours on a roller apparatus and loaded into exposure chambers. A dilution blank was created by combining test sediment with 10% formulated sediment, and an Ambersorb blank was created by adding 10% Ambersorb to formulated sediment. At test termination the sediment was sieved through a series of screens ranging from 250-400 μm to retain the Ambersorb. The Ambersorb was then eluted by loading a column with approximately 7.5g resin and pumping 10 mL of acetone through the column at a rate of 1 mL per minute. Post-column acetone was collected in a 50 mL beaker and evaporated to a final volume of one mL. The final volume was combined with 100 mL clean dilution water to create the eluate sample for toxicity testing with *H. azteca*. The 100 mL water volume was chosen because *H. azteca* are tolerant of 1% acetone, and this maximizes the concentration of contaminants recovered from the resin. The magnitude of toxicity and the concentrations of contaminants in the Ambersorb eluate sample are used in the weight-of-evidence for determining the cause of toxicity. This step associates toxicity of the sediment with contaminants recovered from the original sediment sample, but concentrations of contaminants in the Ambersorb eluate may not reflect the actual sediment or interstitial water concentrations. An Ambersorb elution blank was prepared by performing the above treatments on Ambersorb that had been combined with formulated sediment. A 1% acetone blank was also tested.

• Powdered coconut charcoal (PCC) is pyrolized, activated coconut husk that has been ground to <45 μm (90-96%, Calgon Carbon, Pittsburgh, PA, USA; Ho et al. 2004). Like Ambersorb, PCC is added to sediment to reduce the bioavailability of organic contaminants. Because it provides greater surface area, PCC is more efficient than Ambersorb at sorbing contaminants (Ho et al., 2004). It useful to include both PCC and Ambersorb in solid-phase TIEs, particularly in sediments where toxicity is not reduced

with Ambersorb addition. Relative toxicities of sediments treated with PCC and Ambersorb can provide useful TIE information, particularly when these data are combined with results of Ambersorb eluate tests and chemical analyses of sediment and eluate. Prior to mixing with sediment, the PCC was hydrated with an excess of fresh well water in a 2000 ml Erlenmeyer flask, then vacuum-filtered to form damp slurry. Ten percent (by wet wt) PCC was added to the sediment. PCC-treated sediment was homogenized for 24 h on a Wheaton Roller Apparatus (Wheaton Instruments, Millville, NJ, USA), and then loaded into exposure chambers. A dilution blank was created to account for the possible reduction of sample toxicity that may occur with addition of PCC. A dilution control was created by combining test sediment with the appropriate percentage clean control sediment. A PCC blank was created to account for any toxic effects resulting from adding PCC to test sediment by combining the appropriate percentage PCC with control sediment. All blanks are equilibrated on the sediment roller as previously described.

• SIR-300 (ResinTech, West Berlin, NJ) is a macroporous weak acid cation exchange resin based on the iminodiacetate acid functional group, which has chelating properties for heavy metal ions even in conditions with high calcium concentrations. After preparation, SIR-300 can be mixed into sediment to reduce cationic metal bioavailability (Burgess et al. 2000). Ten percent SIR-300 (wet weight) was added to the sediment in a 500 mL mixing jar. Treated sediment was homogenized for 24 hours on a roller apparatus, and loaded into exposure chambers. A dilution blank was created by combining test sediment with 10% formulated sediment, and an SIR-300 blank was created by adding 10% SIR-300 to formulated sediment. At test termination the sediment was sieved through a series of screens ranging from 250-400 μm to retain the SIR-300. The SIR-300 was then eluted by loading a column with approximately 7.5g resin and pumping 10 mL of 1N hydrochloric acid through the column at a rate of 1 mL per minute. Post-column acid was combined with 100 mL clean dilution water and neutralized to create the eluate sample for toxicity testing with *H. azteca*. An SIR-300 elution blank was prepared by performing the above treatments on SIR-300 that had been combined with formulated sediment. An acid blank was also tested.

• The enzyme carboxylesterase (Sigma-Aldrich, St. Louis, MO) hydrolyzes ester-containing compounds such as pyrethroids to their corresponding acid and alcohol, which are generally not toxic (Wheelock et al. 2004). Carboxylesterase (500x) was added to the overlying water on the day of test initiation, six hours prior to the addition of amphipods. This allowed for interaction between the enzyme and pyrethroids. The enzyme was added based on units of activity. One 'x' of enzyme activity equals 0.0025 units of enzyme per mL of sample, therefore at 500x, 1.25 units per mL were added. Enzyme strength is unique for each lot purchased (Wheelock et al. 2004). To control for the binding of contaminants to the protein base of the enzyme, a separate set of replicates was treated with bovine serum albumin (BSA). Reduction of toxicity by the enzyme, and not the BSA, provides evidence of toxicity due to pyrethroid pesticides. The enzyme and protein treatments were given daily renewals of BSA and carboxylesterase.

• Piperonyl butoxide (Sigma-Aldrich, St. Louis, MO) is a metabolic inhibitor used to block the metabolic activation of acetylcholinesterase-inhibiting organophosphate pesticides (Ankley et al. 1995). It is also a potent synergist of pesticide toxicity, because it inhibits their metabolism (Ware 1989, Kakko et al. 2000). The PBO treatment contained 500 μg/L of PBO in the water overlying the sediment. Decreased toxicity with the addition of PBO suggests the presence of organophosphate pesticides. Increased toxicity with the addition of PBO suggests the presence of pyrethroids.

The following Phase I TIE treatments were performed on a dilution series of water and sediment interstitial water samples (US EPA 1991). Sample concentrations were 0 (treatment blank), 10, 25, 50, and 100%. Treatment blanks consisted of control water that underwent the same manipulation as the sample.

• Baseline – Toxicity test conducted on un-manipulated sample to determine the magnitude of toxicity. Concentrations were chosen to bracket the effect concentration of the sample and might differ from initial test.

• Cation Column – The Cation Column removes cationic metals from the sample. The column was eluted with 1N hydrochloric acid (HCl) and the resulting eluate was tested to determine if substances removed by the column were toxic after being spiked into control water.

• HLB Column – The HLB Column is designed to remove non-polar organic compounds from the sample. In the manipulation, reverse phase liquid chromatography was applied to extract nonionic organic toxicants from the water or interstitial water sample. The HLB column was eluted with methanol and the resulting eluate was tested to determine if substances removed by the column were toxic. Oasis® HLB columns were used for all treatments (Hydrophilic-Lipophilic Balance®, 6 mL, 500 mg, Waters Corporation, Milford, MA, USA). All column treatments followed the manufacturer's suggested generic method for conditioning and loading. The column and pump apparatus was constructed by placing a column in a ring stand clamp, attaching tubing to the outlet of the column, and then passing the tubing through a peristaltic pump. Prior to attachment to the column, the tubing was cleaned by passing 10 mL 1N HCl, 25 mL Nanopure®, 25 mL methanol, and 25 mL Nanopure. After attaching tubing to the columns, they were conditioned by passing 3 mL methanol followed by 5 mL Nanopure. After conditioning, columns were immediately loaded. A separatory funnel was clamped above the column and filled with 100 mL control water. The control water was dripped into the column and pumped through at a rate of 1 mL per minute. After control water had passed through the column, 100 mL of interstitial water was pumped through. Test concentrations were prepared by combining control and sample rinsates (rinsate = HLB-filtered pond water or HLB-filtered sediment interstitial water. Test concentrations were 0, 10, 25, 50, and 100%. After filtering the sample, the columns were eluted by first washing with 4 mL Nanopure, followed by 4 mL of solvent (acetone). Eluate fractions were evaporated to 1 mL and reconstituted in 100 mL control water. Toxicity of the spiked eluates were tested with either *C. dubia* (water) or *H. azteca* (interstitial water), to assess whether toxic concentrations of organic chemicals were recovered from the HLB columns. Test concentrations were prepared by combining reconstituted fractions with control water containing similar concentrations of the appropriate solvent.

• Sequential HLB Cation Column – The two solid-phase extraction columns are used in sequence to determine if toxicity was caused by both metals and organics. Each column is individually eluted to determine if substances removed by the columns were toxic.
• Carboxylesterase – As in the solid-phase treatment, carboxylesterase was added to the sample to hydrolyze pyrethroid pesticides (Wheelock et al. 2004). Bovine serum albumin (BSA) was added in a separate treatment to control for the binding of contaminants to the protein base of the enzyme
• PBO was added to the interstitial water to determine if organophosphate or pyrethroid pesticides were causing toxicity.
• Carboxylesterase/PBO was added to determine if organophosphate or pyrethroid pesticides were causing toxicity

Water exposures were conducted in 20 mL glass scintillation vials (3 replicates) containing 10 mL treated sample and five amphipods (*H. azteca*), or 15 mL treated sample and five daphnids (*C. dubia*). Amphipods were exposed for ten days and daphnids were exposed for four days. Water quality parameters of dissolved oxygen, pH and conductivity were measured using a Hach SensION© selective ion meter with appropriate electrodes; and ammonia was measured using a Hach 2010 spectrophotometer. Temperature was measured using a continuously recording thermograph and thermometer.

Toxicity Data Interpretation

Samples were defined as toxic if the following two criteria were met: 1) there was a significant difference ($p<0.05$) in mean organism response (e.g., percent survival) between a sample and the negative laboratory control, as determined using a separate-variance *t*-test, and 2) the difference in organism response between the sample and control was greater than 20% (Phillips et al. 2001).

TIE data were evaluated by first examining the treatment blanks to determine if sample manipulations added toxic artifacts. Treatment data were then compared to one another using the toxic unit approach for the water samples or simply based on organism

response. Toxic units (TU) were calculated by dividing 100 by the LC50 (as percent sample) calculated from each treatment dilution series. A lower toxic unit value indicates a treatment has been effective in reducing toxicity.

Chemical Analysis

Concentrations of the organophosphate pesticides chlorpyrifos and diazinon were measured using enzyme-linked immunosorbent assays (ELISA, Strategic Diagnostics Inc, Newark, DE). ELISA procedures followed those recommended by Sullivan and Goh (2000). Readings were compared to a 5-point standard curve prepared using standards provided by the manufacturer. Accuracy was determined for each batch using external standards and matrix spikes. All standard measurements were within ± 20% of nominal. Precision was determined by duplicate measurement of one sample per batch. Duplicate coefficients of variation were always less than 20%. Samples were tested without dilution unless necessary. Lowest detectable doses for this procedure were 30 ng/L for diazinon and 50 ng/L for chlorpyrifos. Reporting limits were twice the lowest detectable doses.

Sediment samples were analyzed for organochlorine compounds (U.S. EPA Method 8081), pyrethroids (U.S. EPA Method 1660), and organophosphates (U.S. EPA Method 8141). All analyte identifications were confirmed by gas chromatography-mass spectrophotometer or liquid chromatograph- mass spectrophotometer. Acetone eluates of the Ambersorb and methanol eluates of the HLB treatments were also analyzed for the same three classes of pesticides. Ambersorb eluates were further analyzed using direct injection of the pure solvent into the gas chromatograph.

Quality Assurance

Toxicity testing precision was evaluated by conducting duplicate tests on eleven samples and by evaluating reference toxicity tests in relation to past test performance. Reference toxicant tests were conducted on using the standard protocol on a dilution series of copper for *C. dubia* and cadmium for *H. azteca*. ELISA chemistry precision and accuracy were evaluated through the analysis of external laboratory reference materials

and the measurements of sample duplicates. The quality assurance of the GC/MS chemical analysis was evaluated with the measurement of laboratory blanks, duplicates, standard reference materials, and surrogates.

Toxicity Quality Assurance

All toxicity test controls had acceptable survival based on the criteria set forth in the U.S. EPA protocols. One set of tests was repeated. The *H. azteca* sediment tests conducted as part of the eight Tembladero sampling event and the fifth farm pond sampling event, which were conducted together, had control survival of 76%, compared to the acceptability criterion of 80%. These samples were re-tested, and the repeat test met all quality assurance criteria, with a control response of 94%. Only data from the repeat test were used in subsequent analyses.

The performance of the toxicity test organisms was also evaluated by conducting reference toxicant tests. Reference toxicant tests conducted during this study met all quality assurance criteria, as indicated by control charts showing all results within control limits (Figures 4 through 6).

Figure 4. Toxicity test control chart: C. dubia survival.
Dark lines represent the mean response over a series of tests, the upper control limit and the lower control limit. All tests for this study were within QA/QC control limits.

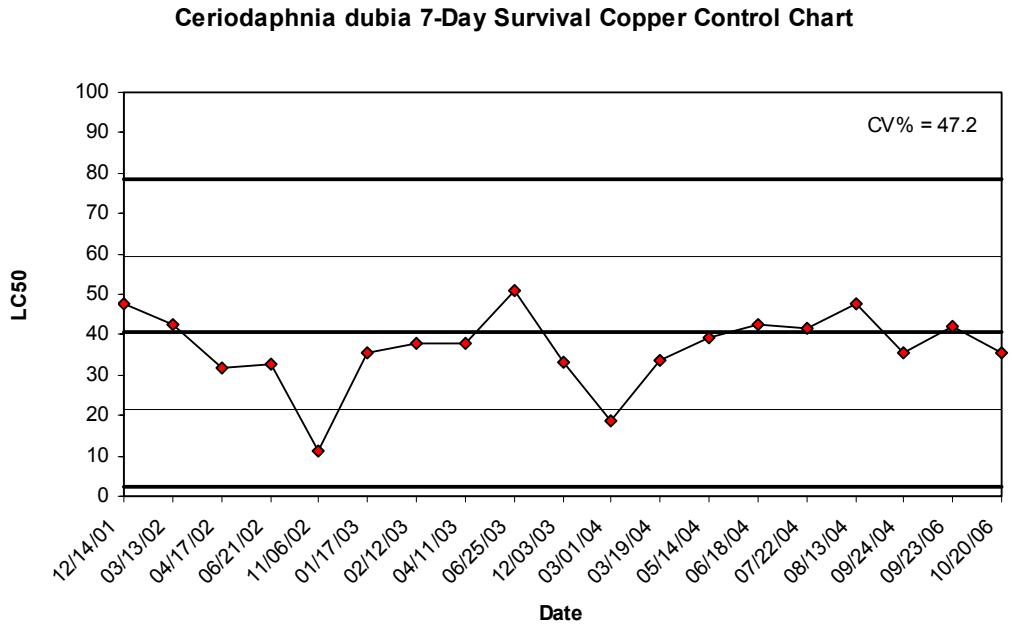


Figure 5. Toxicity test control chart: C. dubia reproduction.
Dark lines represent the mean response over a series of tests, the upper control limit and the lower control limit. All tests for this study were within QA/QC control limits.

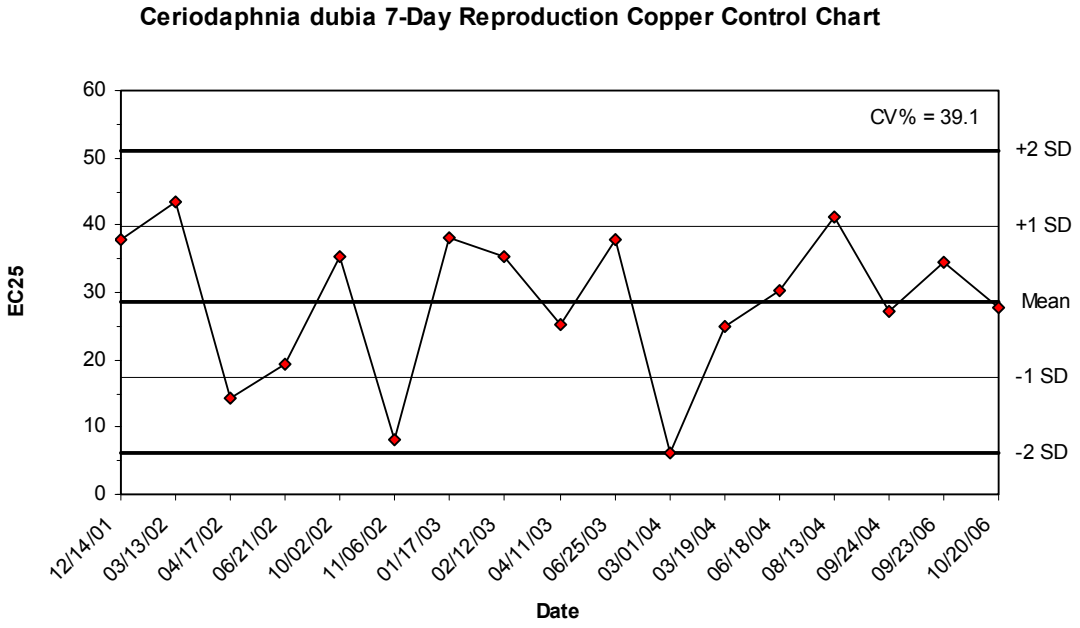
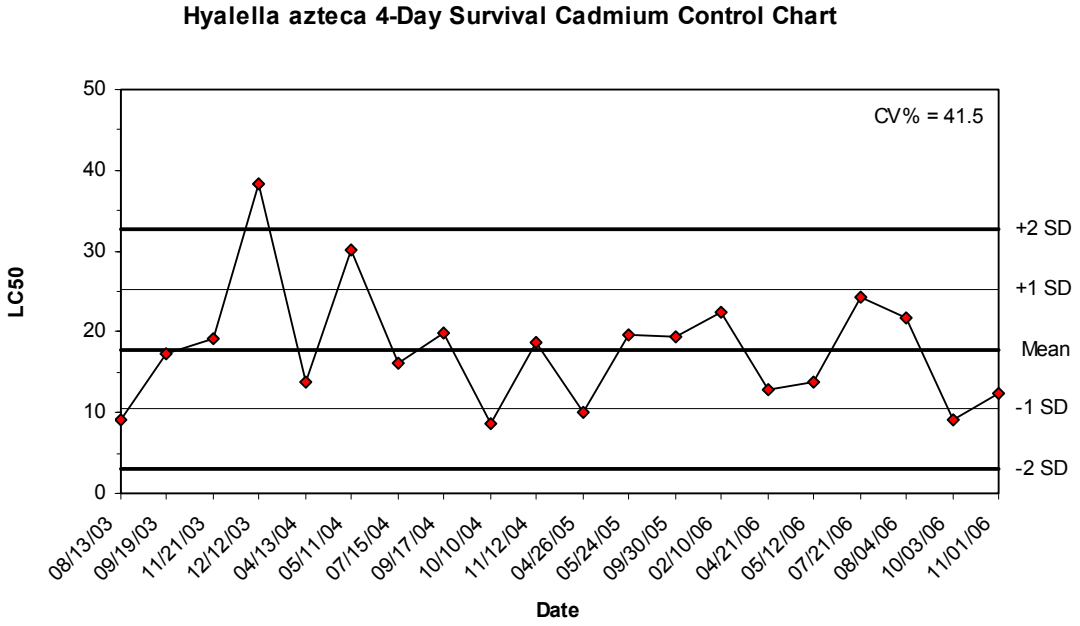


Figure 6. Toxicity test control chart: H. azteca survival.
Dark lines represent the mean response over a series of tests, the upper control limit and the lower control limit. All tests for this study were within QA/QC control limits.



The relative percent differences (RPD) between toxicity test duplicates were below 20% for all samples, except for those in which low survival or low growth values exaggerated the relative differences (Table 1). In the first *H. azteca* sediment test for Tembladero, both samples were similarly toxic with 9% and 14% survival, and the RPD was 56%. The growth results for the same samples also showed similar toxicity, and had an RPD of 85%. A set of *C. dubia* duplicate samples both showed similarly high reproductive toxicity, and had an RPD greater of 29%.

Table 1. Toxicity test QA results for duplicate samples.

Run	Date	Station	Organism	Endpoint	Baseline	Duplicate	Relative Percent Difference
Tembladero							
2	7/21/06	DCR-001	*C. dubia*	Survival	0	0	0
2	7/21/06	DCR-001	*C. dubia*	Reproduction	0	0	0
3	8/24/06	TEM-MOL	*H. azteca*	Survival	9	14	56
3	8/24/06	TEM-MOL	*H. azteca*	Growth	0.125	0.231	85
6	10/4/06	DCR-001	*C. dubia*	Survival	89	100	12
6	10/4/06	DCR-001	*C. dubia*	Reproduction	17	19	12
6	10/4/06	DCR-001	*H. azteca*	Survival	84	84	0
8	11/1/06	DCR-001	*C. dubia*	Survival	100	100	0
8	11/1/06	DCR-001	*C. dubia*	Reproduction	23	19	17
8	11/1/06	DCR-001	*H. azteca*	Survival	76	76	0
Gabilan							
5	10/27/06	G-09 A	*C. dubia*	Survival	0	0	0
5	10/27/06	G-09 A	*C. dubia*	Reproduction	0	0	0
5	10/27/06	G-09 B	*C. dubia*	Survival	0	0	0
5	10/27/06	G-09 B	*C. dubia*	Reproduction	0	0	0
5	10/27/06	SV-03 A	*C. dubia*	Survival	80	90	0
5	10/27/06	SV-03 A	*C. dubia*	Reproduction	7	5	29
5	10/27/06	SV-03 C	*C. dubia*	Survival	0	0	0
5	10/27/06	SV-03 C	*C. dubia*	Reproduction	0	0	0

ELISA QA

Enzyme-linked immunosorbent assays (ELISAs) were used as a rapid measure of diazinon and chlorpyrifos concentrations in test samples. These tests had good agreement among duplicate samples (Table 2), and external standard measurements were within control limits for chlorpyrifos (Figure 7), and for all but one measurement of diazinon (Figure 8).

Table 2. QA results for duplicate samples analyzed by ELISA.
Enzyme-linked immunosorbent assays to measure concentrations of the organophosphate pesticides diazinon and chlorpyrifos.

Run	Date	Station	Organophosphate	Baseline (ng/L)	Duplicate (ng/L)	Relative Percent Difference
Tembladero						
2	7/21/06	DCR-001	Chlorpyrifos	<RL	<RL	0
2	7/21/06	DCR-001	Diazinon	256	237	7
4	9/7/06	DCR-001	Chlorpyrifos	ND	ND	NA
4	9/7/06	DCR-001	Diazinon	145	130	10
6	10/4/06	DCR-001	Chlorpyrifos	ND	ND	NA
6	10/4/06	DCR-001	Diazinon	103	108	5
8	11/1/06	DCR-001	Chlorpyrifos	ND	ND	NA
8	11/1/06	DCR-001	Diazinon	ND	ND	NA
Gabilan						
5	10/27/06	G-09 A	Chlorpyrifos	461	531	15
5	10/27/06	G-09 A	Diazinon	<RL	<RL	0
5	10/27/06	G-09 B	Chlorpyrifos	330	299	9
5	10/27/06	G-09 B	Diazinon	<RL	<RL	0
5	10/27/06	SV-03 A	Chlorpyrifos	ND	ND	NA
5	10/27/06	SV-03 A	Diazinon	<RL	<RL	0
5	10/27/06	SV-03 B	Chlorpyrifos	ND	ND	NA
5	10/27/06	SV-03 B	Diazinon	189	170	10

Figure 7. Control chart for ELISA measurement of external standards for chlorpyrifos.

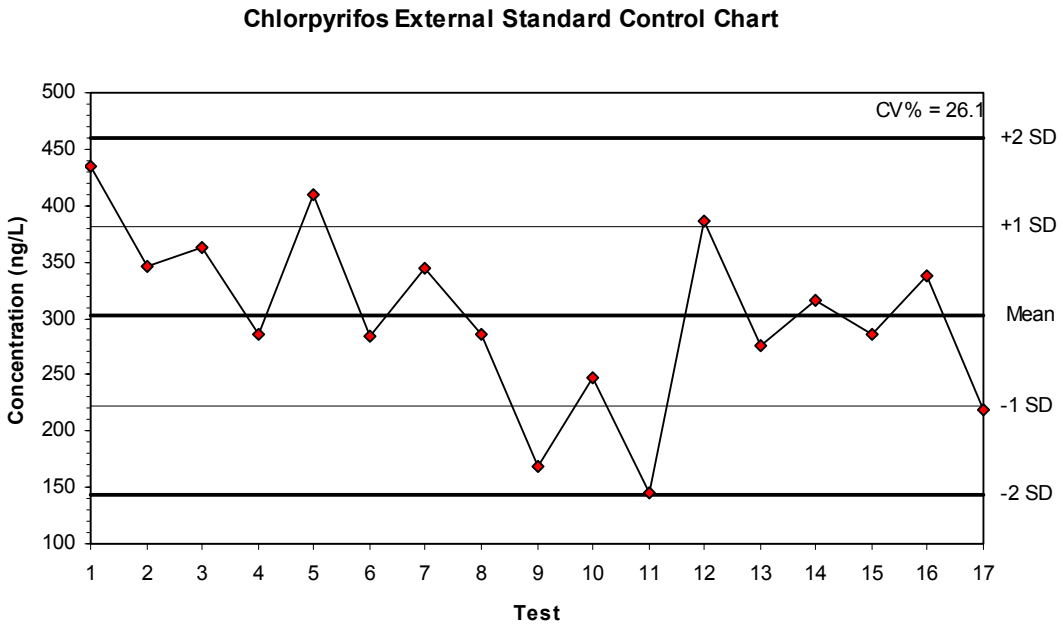
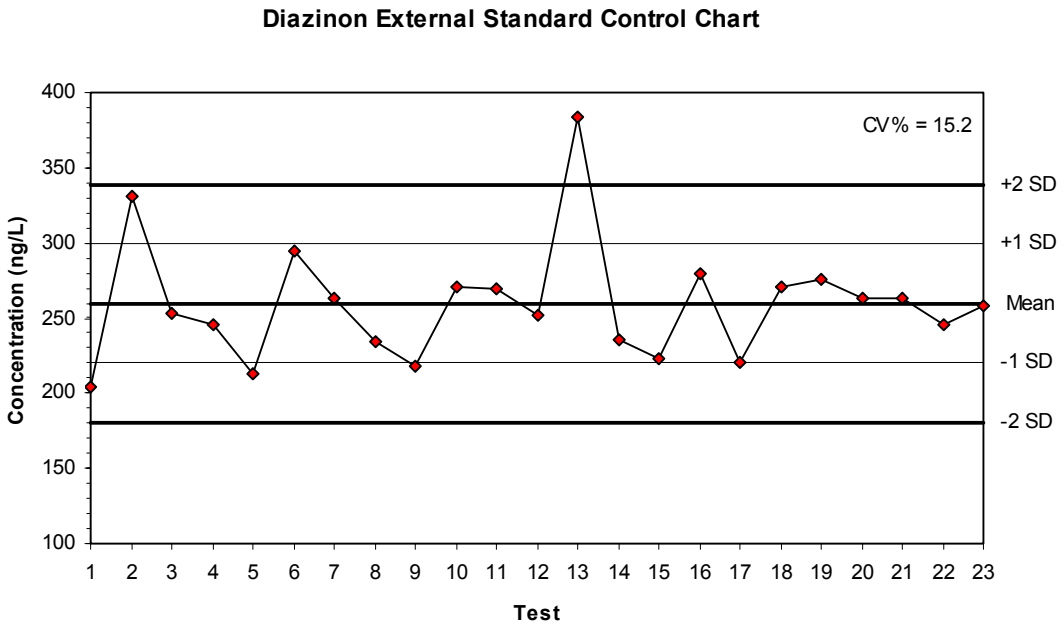


Figure 8. Control chart for ELISA measurement of external standards for diazinon.



RESULTS and DISCUSSION

Tembladero constructed wetland system

Eight surveys were conducted from July to November, during the irrigation season. Pumping rates were changed every two weeks, to produce eight different flow regimes, each with its own characteristic residence time. Flow regimes were changed in random order (Table 3). Relationships between residence time and treatment effectiveness are considered in a subsequent section, but it may be useful to keep the residence times in mind when considering the following results.

Table 3. Residence times for water passing through the channel portion of the vegetated treatment system at Tembladero Slough, and corresponding sampling dates.

Sampling Date 2006	Estimated Residence Time (days)
July 5	5.92
July 19	3.95
August 23	0.94
September 6	2.72
September 20	0.90
October 4	2.85
October 18	8.84
November 1	1.75

During the first survey, there was 0% survival in toxicity tests of water samples from the Slough itself (Tem-Mol), the VTS inlet (DCR-001), and the channel stations (DCR-002 and DCR-003; Table 4a). There was some reproductive output in the DCR-003 sample (before the adult *C. dubia* died), and survival increased to 100% at the wetland outlet (Table 4a; Figure 1).

Concomitant with increasing survival was a decrease in organophosphate (OP) pesticide concentrations at stations further down the system (Figure 9). This decline was driven mainly by lower diazinon concentrations at downstream stations. Diazinon concentrations were near the median lethal concentration (LC50; Table 5) at the inlet station (DCR-001), and because organophosphate toxicity is additive (Bailey et al. 1997), it is likely that toxicity observed during this survey was caused primarily by the organophosphates.

Table 4a. Toxicity, ELISA measurements of diazinon and chlorpyrifos, and conductivity (EC) summary for Tembladero constructed wetland surveys 1 – 4.

			Ceriodaphnia (100%)				Ceriodaphnia (dilution)			Hyalella Water		Hyalella Sediment				Chlor.	Diaz.	EC
No.	Date	Station	Mean % Survival	Mean # Neonates	SD # Neonates	LT50	Mean % Survival	Mean # Neonates	SD # Neonates	Mean % Survival	SD % Survival	Mean % Survival	SD % Survival	Mean Growth (mg/ind)	SD Growth (mg/ind)	ng/L	ng/L	uS/cm
1	7/7/06	TEM-MOL	0*	0*	0*	1.4										<RL	451	2610
1	7/7/06	DCR-001	0*	0*	0*	1.3										<RL	438	4230
1	7/7/06	DCR-002	0*	0*	0*	2.2										<RL	392	2550
1	7/7/06	DCR-003	0*	4*	1.6*	4.4										<RL	112	2370
1	7/7/06	DCR-004	100	17	6.6	NA										<RL	288	2490
2	7/21/06	TEM-MOL	0*	3*	3.1	4.5										<RL	280	3610
2	7/21/06	DCR-001	0*	0*	0	1.3										<RL	256	5630
2	7/21/06	DCR-003	20*	14	8.2	5.8										<RL	339	2760
2	7/21/06	DCR-004	70	17	13.1	NA										<RL	194	2730
3	8/24/06	TEM-MOL										9*	11	0.125	0.096	ND	298	2180
3	8/24/06	DCR-001										83	9	0.167	0.043	ND	<RL	9480
3	8/24/06	DCR-002														ND	198	7440
3	8/24/06	DCR-003										90	5	0.211	0.048	ND	241	8780
3	8/24/06	DCR-004														ND	214	8670
4	9/7/06	TEM-MOL														ND	219	2650
4	9/7/06	DCR-001														ND	145	6850
4	9/7/06	DCR-003														ND	205	7910
4	9/7/06	DCR-004														ND	166	6750

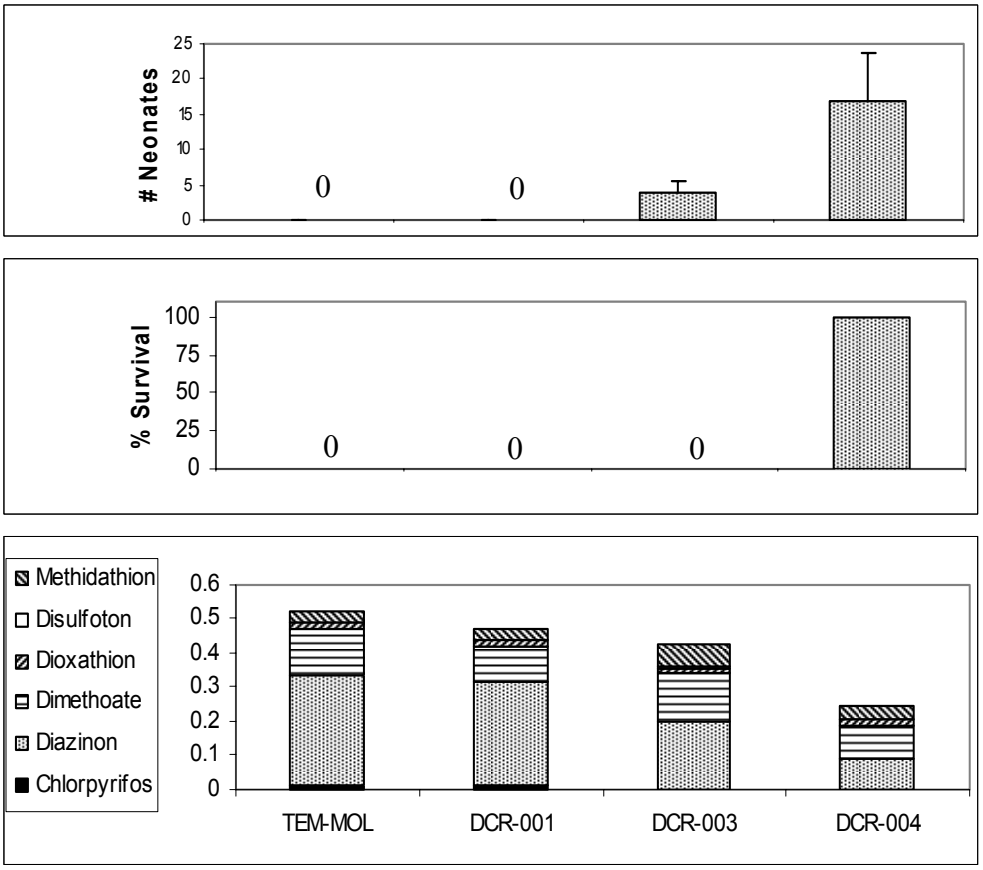
* Significantly different from the control and endpoint less than the toxicity threshold

Table 4b. Toxicity, ELISA, and conductivity summary for Tembladero constructed wetland surveys 5 – 8.

			Ceriodaphnia (100%)				Ceriodaphnia (dilution)			Hyalella Water		Hyalella Sediment				Chlor.	Diaz.	EC
No,	Date	Station	Mean % Survival	Mean # Neonates	SD # Neonates	LT50	Mean % Survival	Mean # Neonates	SD # Neonates	Mean % Survival	SD % Survival	Mean % Survival	SD % Survival	Mean Growth (mg/ind)	SD Growth (mg/ind)	ng/L	ng/L	uS/cm
							25%											
5	9/22/06	TEM-MOL					100	13	4.9	2*	4	24*	13	0.128	0.055	ND	247	2890
5	9/22/06	DCR-001					100	17	3.6	94	9	84	7	0.285	0.031	ND	148	9110
5	9/22/06	DCR-002					100	18	5.2	88	4	81	14	0.257	0.036	ND	<RL	8370
5	9/22/06	DCR-003					100	20	4.1	92	13	90	5	0.359	0.034	ND	<RL	10020
5	9/22/06	DCR-004					100	20	6.1	98	4					ND	<RL	10100
							30%											
6	10/4/06	TEM-MOL					100	18	3.0	66*	21					ND	110	4150
6	10/4/06	DCR-001					89	17	5.2	84	9					ND	103	4570
6	10/4/06	DCR-003					100	20	1.0	96	5					ND	<RL	7860
6	10/4/06	DCR-004					100	20	3.4	98	4					ND	<RL	7770
							20%											
7	10/18/06	TEM-MOL					100	23	3.6	88	8					ND	103	3530
7	10/18/06	DCR-001					100	20	6.8	90	12					ND	ND	12850
7	10/18/06	DCR-002					100	26	7.3	94	5					ND	ND	5600
7	10/18/06	DCR-003					100	27	10.1	90	6					ND	ND	5740
7	10/18/06	DCR-004					100	26	6.6	96	5					ND	ND	6530
							15%											
8	11/1/06	TEM-MOL					100	23	2.2	72	16	60*	13	0.064*	0.038	ND	<RL	4800
8	11/1/06	DCR-001					100	23	2.7	76	11	84	17	0.178	0.027	ND	ND	13560
8	11/1/06	DCR-002										95	9	0.206	0.027	ND	ND	12520
8	11/1/06	DCR-003					100	22	1.9	92	4	84	15	0.185	0.024			
8	11/1/06	DCR-004					100	22	4.4	94	9					ND	ND	12010

* Significantly toxic: statistically significant difference from the control and endpoint less than the toxicity threshold.

Figure 9. Organophosphate pesticide concentrations and *C. dubia* survival and reproduction in samples from the constructed wetland, July 5, 2006. Concentrations are in µg/L. TEM-MOL is Tembladero Slough surface water, DCR-001 is the channel inlet, DCR-003 is the channel outlet, and DCR-004 is the wetland outlet. See Figure 1 for system diagram.



Organochlorine pesticide concentrations were progressively lower at sites further down the system (Figure 10). The combined organochlorine concentrations were dominated by dacthal, an alkyl phthalate herbicide with low toxicity to fish and invertebrates (Table 5). Dacthal is in current use, with greatest statewide applications on broccoli, cauliflower, and other irrigated row crop vegetables. The biological effects of herbicides were not measured in this study. Legacy organochlorine pesticides, such as DDT, were measured at low concentrations throughout the system (Tables 5 & 6). Declines in organochlorines here are likely related to settling out of the suspended particles to which they bind in the water column, as discussed at the end of this section. This was the only survey in which organochlorines were measured.

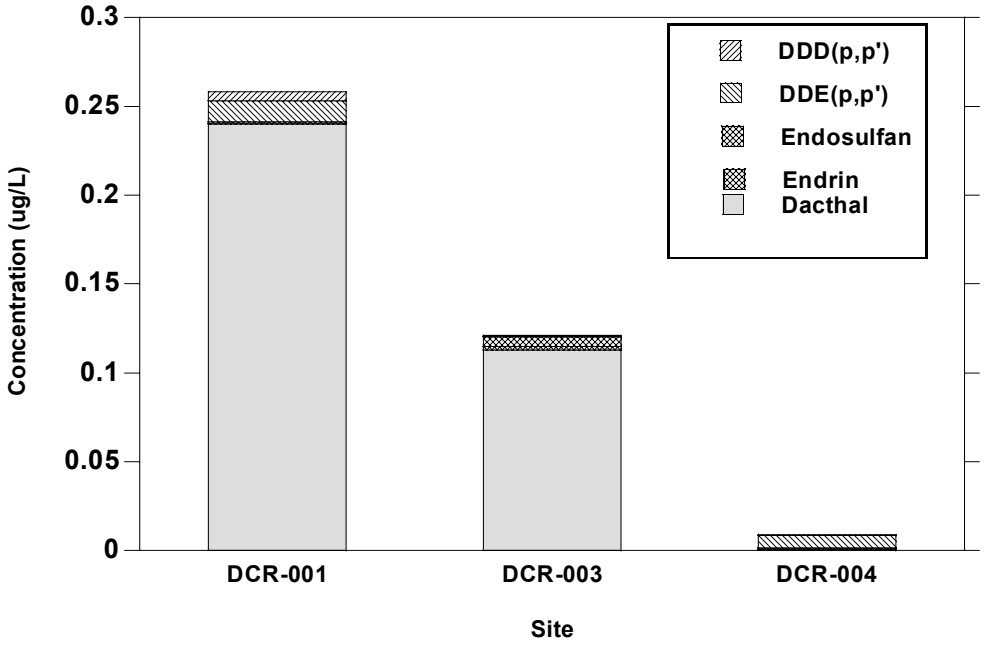
Table 5. Maximum concentrations measured in this study compared to median lethal concentrations (LC50s) or water quality guidelines.

Chemical	Maximum Concentration Observed (ug/L)			Threshold Concentration (ug/L)	Threshold Type	Reference
	Temb	G-09	SV-03			
Chlorpyrifos	0.016	**0.762 ***	0.021	0.053	96-h LC50	1
Diazinon	**0.322 ***	0.041	**9.62 ***	0.32	96-h LC51	1
Dimethoate	0.52	nd	8.4	600	48-h LC50	9
Dioxathion	0.91	2.4	nd	na	24-h LC50	na
Methidathion	0.666	nd	nd	6.4	48-h EC50	10
Bifenthin	nd	0.007	nd	0.142	48-h LC50	8
Cyfluthrin	0.007	0.04	0.017	0.344	48-h LC50	8
Cypermethrin	0.002	0.02	**0.608 ^**	0.683	48-h LC50	8
L-Cyhalothrin	nd	nd	0.002	0.200	48-h LC50	8
Permethrin	nd	0.998 *	0.033	0.250	48-h LC50	8
Carbaryl	nd	nd	0.059	1.50	ALB	6
Carbofuran	nd	nd	0.35	2.23	LC50	2
Methomyl	nd	0.15	0.44	47	LC50	3
Dacthal	0.24	nd	0.055	14300	Acute WQC	5
DDD(p,p')	0.005	nd	0.02	0.170	LC50	4
DDE(p,p')	0.012	0.008	0.187	1.4	LC50	4
DDT(p,p')	nd	0.004	**0.094 ***	0.07	LC50	4
Dieldrin	nd	nd	0.036	7.6	LC50	4
Endosulfan	0.005	0.023	nd	0.056	4-d Ave	7
Endrin	nd	nd	0.027	0.036	Chronic WQC	5

References for Table 5:
1 *Ceriodaphnia dubia*, Bailey et al., 1997
2 *Ceriodaphnia dubia*, Bailey *et al*., 1996
3 *Gammarus italicus*, Pantani et al., 1997
4 *Hyalella azteca*, Phipps et al., 1995
5 Ambient Water Quality Criteria Recommendations, USEPA 2000
6 Aquatic Life Benchmarks, USEPA 2007
7 California Toxics Rule, cited by Marshack 2007
8 *Ceriodaphnia dubia*, Wheelock et al. 2004
9 *Daphnia magna*, Beusen and Neven, 1989
10 *Daphnia magna* (immobility effect), USEPA, 2000

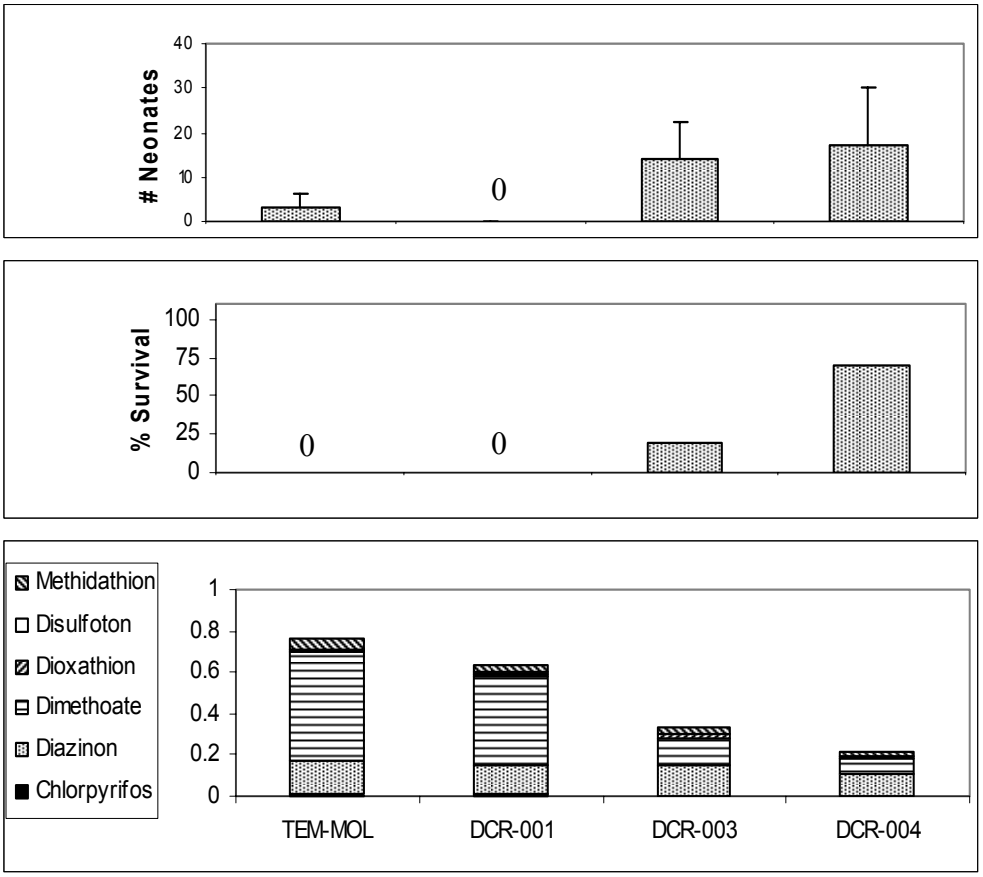
Carbamate pesticides were not detected in water samples from the first survey, and pyrethroid pesticides were detected only at low concentrations near their detection limits (Tables 5 & 6).

Figure 10. Organochlorine pesticide concentrations in samples from the Tembladero Slough constructed wetland, July 5, 2006. DCR-001 is the channel inlet, DCR-003 is the channel outlet, and DCR-004 is the wetland outlet. See Figure 1 for system diagram.



In the second survey, OP pesticides again were found at lower concentrations at stations further down the system. This trend was driven largely by dimethoate. Since this compound is about 200 times less acutely toxic to invertebrates than diazinon, it is unlikely that dimethoate was solely responsible for the observed toxicity, but the combined concentrations of all OPs may have contributed. *C. dubia* survival was 0% in the Slough and at the channel inlet, but was higher at stations further down the system (Figure 11). Carbamate pesticides were not detected, and pyrethroid pesticides were detected only at low concentrations near their detection limits.

Figure 11. Organophosphate pesticide concentrations and *C. dubia* survival and reproduction in samples from the constructed wetland, July 19, 2006. Concentrations are in µg/L. TEM-MOL is Tembladero Slough surface water, DCR-001 is the channel inlet, DCR-003 is the channel outlet, and DCR-004 is the wetland outlet. See Figure 1 for system diagram.



Water collected in the third and fourth surveys (Figures 12 and 13) had high conductivity, and all *C. dubia* test organisms died as a result. These tests were not repeated because the pumping regime had changed in the interim, but all subsequent tests were conducted with both *C. dubia* and *H. azteca* (see Caveats section in the Introduction). Again, carbamate pesticides were not detected, and pyrethroid pesticides were detected only at low concentrations. In contrast to the first two surveys, trends with OP concentration and station location were not apparent in these surveys. As mentioned in the Introduction, the likely variable pesticide concentrations in the inputs, and the fact that pulses of water were not tracked through the system make it difficult to directly compare concentrations between stations in terms of treatment effects.

Figure 12. Organophosphate pesticide concentrations in samples from the constructed wetland, August 23, 2006. Concentrations are in µg/L. DCR-001 is the channel inlet, DCR-003 is the channel outlet, and DCR-004 is the wetland outlet. See Figure 1 for system diagram.

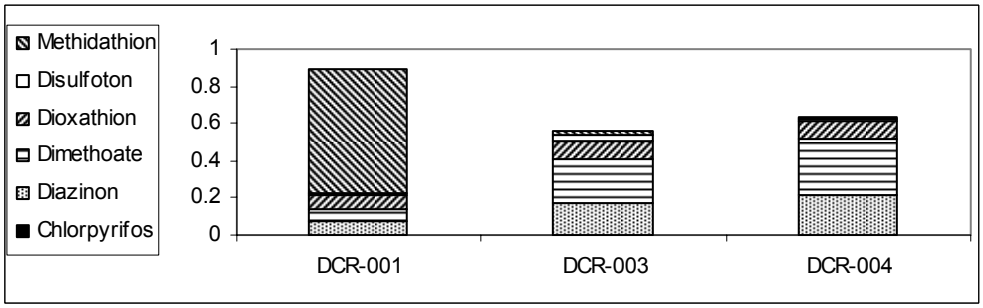
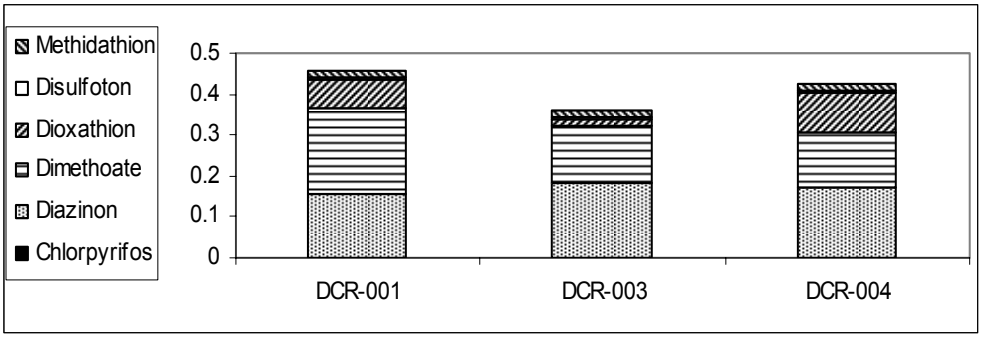


Figure 13. Organophosphate pesticide concentrations in samples from the constructed wetland, September 6, 2006. Concentrations are in µg/L. DCR-001 is the channel inlet, DCR-003 is the channel outlet, and DCR-004 is the wetland outlet. See Figure 1 for system diagram.

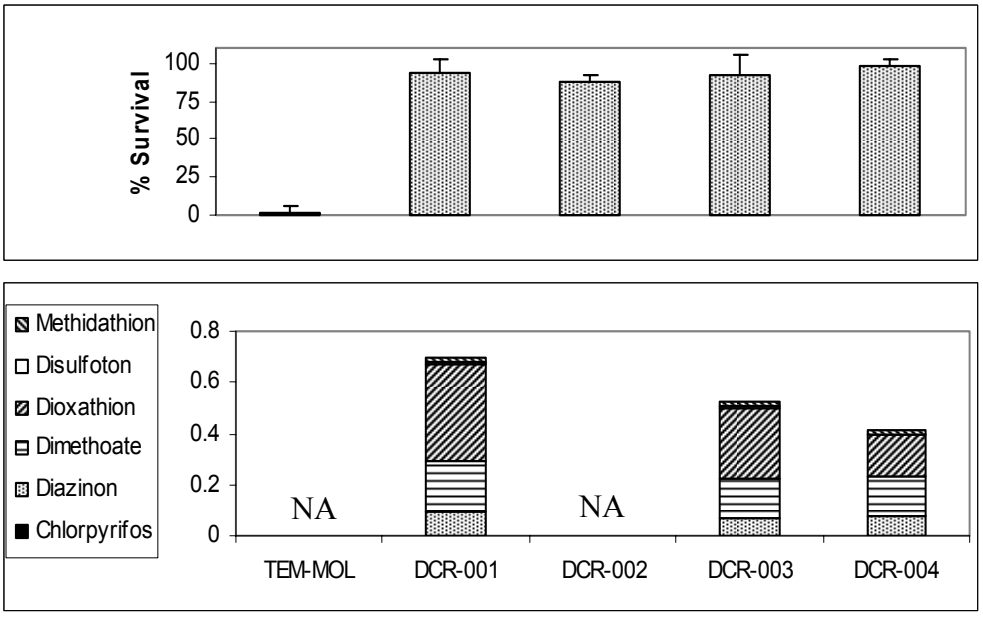


Although *C. dubia* toxicity data were not available, sediments were collected during the August survey, and *Hyalella azteca* sediment toxicity test results demonstrated toxicity in the Slough (Tem-Mol), but not in any samples of settled sediment from the constructed wetland (stations DCR-001 and DCR-003; Table 4). Sediment chemical analyses indicated that concentrations of most detected chemicals were much greater in the Tem-Mol sample than in samples of sediment that settled out at the VTS channel stations (Table 7), but no chemicals were apparently elevated to concentrations that could be solely implicated as the causes of Tem-Mol sediment sample toxicity.

In the fifth survey (Figure 14), total OP pesticide concentrations were lower at stations further down the system, and this decrease is driven primarily by dioxathion. Dioxathion is a cholinesterase inhibiting insecticide that would be expected to add to the toxicity of similar OPs like diazinon and chlorpyrifos. This pesticide is used infrequently, often for landscaping.

Toxicity was observed during this survey in the Slough sample. Because of high conductivities, this toxicity was measured with *H. azteca*, and no other samples were

Figure 14. Organophosphate pesticide concentrations and *H. azteca* survival in samples from the constructed wetland September 20, 2006. Concentrations are in µg/L. TEM-MOL is Tembladero Slough surface water, DCR-001 is the channel inlet, DCR-002 is midway down the channel, DCR-003 is the channel outlet, and DCR-004 is the wetland outlet. See Figure 1 for system diagram. NA = not analyzed.

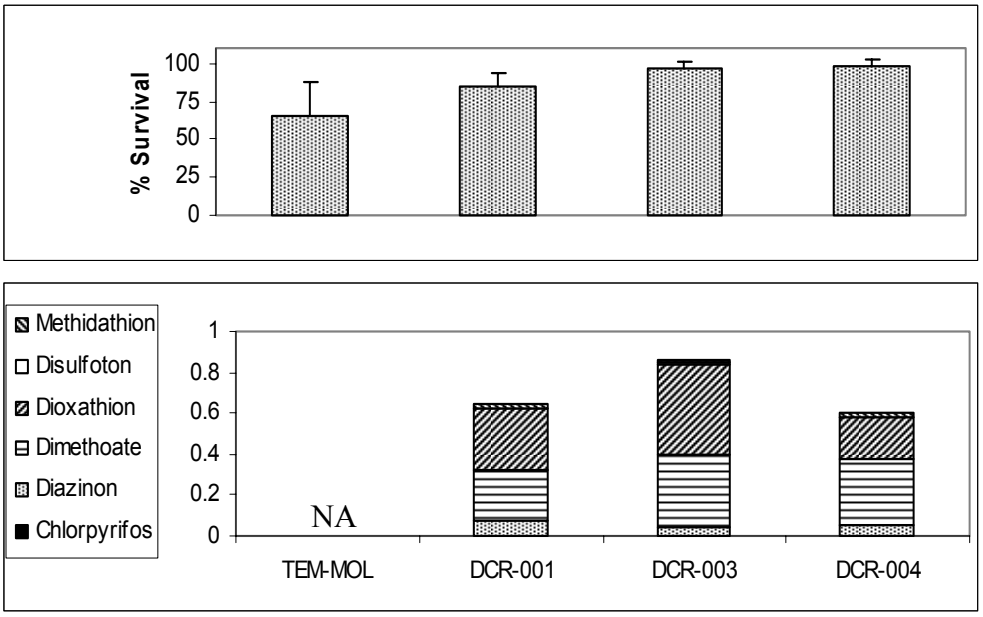


toxic to the amphipod (Figure 14). To compensate for the high conductivities measured *C. dubia* tests were conducted with samples diluted to 25% strength, and no toxicity was observed in these diluted samples.

Sediment collected from the Slough during this survey was toxic to *Hyalella azteca,* but samples of sediment that settled into trays in the channel were not toxic (Table 4). As with the August sediment samples, chemical concentrations were higher in the Slough sample than in the channel samples(Table 7), but no single chemical exceeded toxicity thresholds.

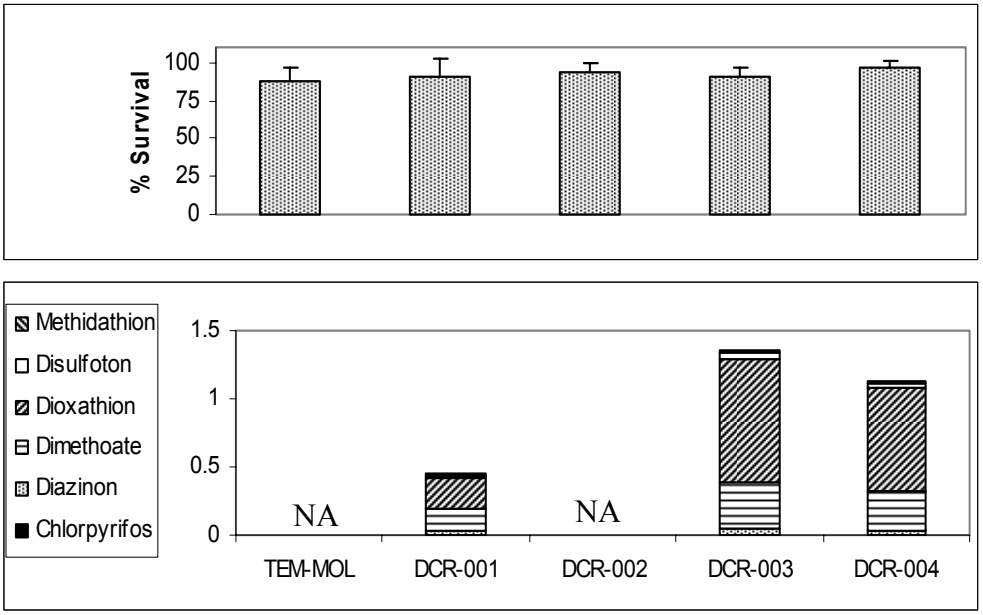
In the sixth survey (Figure 15), there was no apparent trend relating pesticide concentration with station location. Surface water taken directly from the Slough was toxic to *H. azteca*, and amphipod survival was slightly depressed in channel inlet water. Survival was near 100% at the outlets to the channel and wetland. *C. dubia* tests were conducted using samples diluted to 30% strength, and no significant toxicity was observed.

Figure 15. Organophosphate pesticide concentrations and *H. azteca* survival in samples from the constructed wetland October 4, 2006. . Concentrations are in µg/L. TEM-MOL is Tembladero Slough surface water, DCR-001 is the channel inlet, DCR-003 is the channel outlet, and DCR-004 is the wetland outlet. See Figure 1 for system diagram. NA = not analyzed.



Fluctuations in dioxathion and dimethoate drove the total OP concentrations in the seventh survey (Figure 16), and there was no apparent trend relating pesticide concentration with station location. *C. dubia* tests were conducted with samples diluted to 20% strength, and *H. azteca* water tests were conducted with full strength samples. No toxicity was observed.

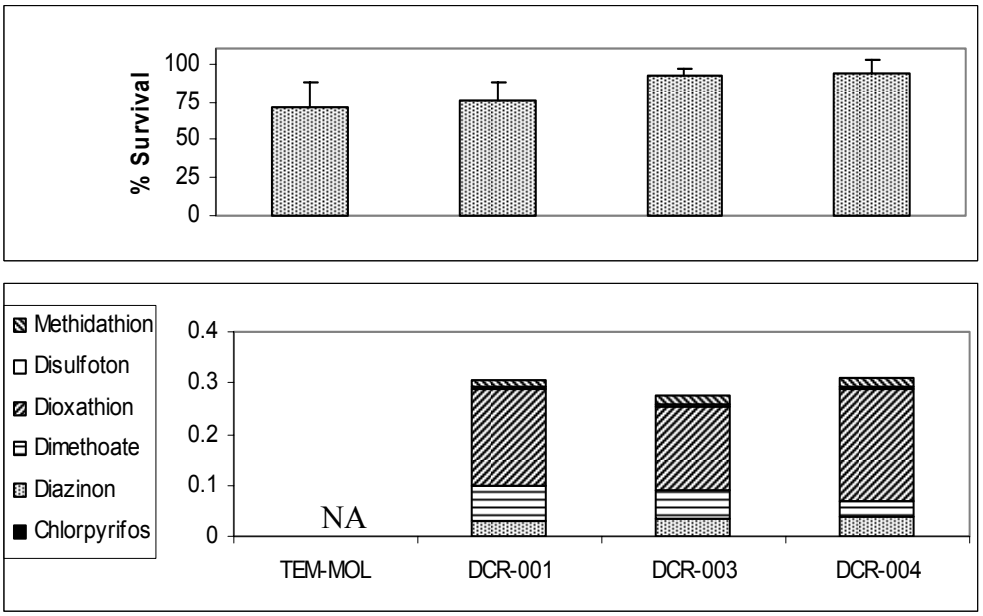
Figure 16. Organophosphate pesticide concentrations and *H. azteca* survival in samples from the constructed wetland October 18, 2006. Concentrations are in µg/L. TEM-MOL is Tembladero Slough surface water, DCR-001 is the channel inlet, DCR-002 is midway down the channel, DCR-003 is the channel outlet, and DCR-004 is the wetland outlet. See Figure 1 for system diagram. NA = not analyzed.



OP pesticide concentrations were similar in samples from the three channel stations measured in the eighth survey (Figure 17). Dioxathion again had the highest concentrations. Amphipod survival was reduced to 72 and 76 %, respectively, in samples from the Slough and the channel inlet, though this decrease was not statistically significant. Survival was higher in samples from the downstream stations. *C. dubia* tests were conducted with samples diluted to 20% strength, and no toxicity was observed.

Sediment collected from the Slough during this survey was toxic to *Hyalella azteca,* but samples of sediment that settled into trays in the channel were not toxic (Table 3). Sediment concentrations of organochlorine pesticides generally decreased with distance down the channel (Table 7). Organophosphates were not detected in the sediments, and pyrethroids were measured at low concentrations.

Figure 17. Organophosphate pesticide concentrations and *H. azteca* survival in samples from the constructed wetland November 1, 2006. Concentrations are in µg/L. TEM-MOL is Tembladero Slough surface water, DCR-001 is the channel inlet, DCR-003 is the channel outlet, and DCR-004 is the wetland outlet. See Figure 1 for system diagram. NA = not analyzed.



One objective of this study was to test hypotheses about pesticide reduction in the treatment systems. For the Tembladero Slough treatment systems, paired-sample separate-variance t-tests were conducted comparing concentrations measured at the inlet (DCR-001) and outlet (DCR-004) of the entire system. There were no statistically significant reductions for total organochlorines, or for any of the individual chamicals for which there were sufficient data for analysis, including diazinon, dimethoate, dioxathion, or total organophosphates.

Table 6a. Summary of measured water concentrations of all chemicals detected in samples from the Tembladero Slough VTS.

				July 5				July 19				Aug 23			Sept 6			
Organophosphates		**MDL**	**RL**	**TEM-MOL**	**DCR-001**	**DCR-003**	**DCR-004**	**DCR-001**	**DCR-001 D**	**DCR-003**	**DCR-004**	**DCR-001**	**DCR-003**	**DCR-004**	**DCR-001**	**DCR-003**	**DCR-004**	**LCS Recovery**
Chlorpyrifos	ug/L	0.003	0.005	0.016	0.015	ND	ND	0.015	0.012	ND	ND	ND	ND	ND	ND	ND	ND	65.8-106
Diazinon	ug/L	0.005	0.020	0.322	0.302	0.198	0.087	0.160	0.140	0.152	0.105	0.073	0.173	0.210	0.152	0.183	0.172	82.2-112
Dimethoate	ug/L	0.030	0.050	0.135	0.104	0.141	0.100	0.520	0.425	0.128	0.072	0.069	0.234	0.300	0.210	0.140	0.133	16.7-107
Dioxathion	ug/L	0.030	0.050	ND	ND	ND	ND	ND	ND	ND	ND	0.071	0.102	0.103	0.073	nd	0.096	75.6-112
Disulfoton	ug/L	0.010	0.050	ND	ND	ND	ND	ND	ND	ND	ND	0.013	0.030	0.010	ND	ND	ND	44.9-95.8
Methidathion	ug/L	0.030	0.050	0.030	0.030	0.067	0.039	0.050	0.042	0.030	ND	0.666	ND	ND	ND	ND	ND	77.5-110
Triphenyl phosphate	Percent Surrogate Recovery			104	108	97.2	92.9	125	111	113	108	106	108	110	99.5	99.7	104	NA
Pyrethroids																		
Cyfluthrin	ug/L	0.002	0.004	0.003	0.003	0.004	0.007	0.003	0.002	0.003	0.004	ND	ND	ND	ND	ND	ND	68.7-100
Cypermethrin	ug/L	0.002	0.004	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	73.3-104
Dibromooctafluorobiphenyl	Percent Surrogate Recovery			98.8	98.7	103	88.7	90.5	90.3	87.6	89.3	92.2	94.1	95.1	76.9	83.4	91.3	NA
Organochlorines																		
Dacthal	ug/L	0.001	0.002	0.218	0.24	0.113	ND	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	83.6-93.4
DDD(p,p')	ug/L	0.001	0.002	0.004	0.005	ND	ND	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	79-86
DDE(p,p')	ug/L	0.001	0.002	0.008	0.012	ND	0.007	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	79.3-90.1
Endosulfan sulfate	ug/L	0.001	0.002	ND	ND	0.005	ND	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	49.2-53.7
Endrin aldehyde	ug/L	0.002	0.005	ND	ND	0.002	ND	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	79.5-86.9
Dibromooctafluorobiphenyl	Percent Surrogate Recovery			100	94.6	99.2	98.2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Table 6b. Summary of measured water concentrations of all chemicals detected in samples from the Tembladero Slough VTS.

				Sept 20			Oct 4			Oct 18			Nov 1			
Organophosphates		**MDL**	**RL**	**DCR-001**	**DCR-003**	**DCR-004**	**DCR-001**	**DCR-003**	**DCR-004**	**DCR-001**	**DCR-003**	**DCR-004**	**DCR-001**	**DCR-003**	**DCR-004**	**LCS Recovery**
Chlorpyrifos	ug/L	0.003	0.005	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	65.8-106
Diazinon	ug/L	0.005	0.020	0.091	0.070	0.075	0.074	0.042	0.052	0.035	0.048	0.035	0.027	0.034	0.037	82.2-112
Dimethoate	ug/L	0.030	0.050	0.204	0.153	0.158	0.248	0.354	0.322	0.149	0.330	0.280	0.072	0.054	0.031	16.7-107
Dioxathion	ug/L	0.030	0.050	0.378	0.278	0.157	0.300	0.444	0.204	0.234	0.910	0.766	0.187	0.164	0.220	75.6-112
Disulfoton	ug/L	0.010	0.050	ND	ND	ND	ND	ND	ND	0.018	0.05	0.038	ND	ND	ND	44.9-95.8
Methidathion	ug/L	0.030	0.050	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	77.5-110
Triphenyl phosphate	Percent Surrogate Recovery			104	108	107	117	105	76.6	77.6	86.3	91.2	109	106	105	101
Pyrethroids																
Cyfluthrin	ug/L	0.002	0.004	0.003	0.003	0.004	ND	ND	ND	ND	ND	ND	ND	ND	ND	68.7-100
Cypermethrin	ug/L	0.002	0.004	ND	0.002	0.002	ND	ND	ND	ND	ND	ND	ND	ND	ND	73.3-104
Dibromooctafluorobiphenyl	Percent Surrogate Recovery			98.8	98.7	97.5	101	109	99.2	90.5	97.3	82.4	104	89.7	109	101
Organochlorines																
Dacthal	ug/L	0.001	0.002	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	83.6-93.4
DDD(p,p')	ug/L	0.001	0.002	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	79-86
DDE(p,p')	ug/L	0.001	0.002	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	79.3-90.1
Endosulfan sulfate	ug/L	0.001	0.002	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	49.2-53.7
Endrin aldehyde	ug/L	0.002	0.005	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	79.5-86.9
Dibromooctafluorobiphenyl	Percent Surrogate Recovery			100	94.6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Table 7a. Summary of measured sediment concentrations of all chemicals detected in samples from the Tembladero Slough VTS.

				August 23				September 20				November 1				
		MDL	RL	TEM-MOL	DCR-001	DCR-003	DCR-001D	TEM-MOL	DCR-001	DCR-003	DCR-004	TEM-MOL	DCR-001	DCR-003	DCR-004	LCS Recovery
Organochlorines																
Aldrin	ng/g	0.181-0.650	1.39-5.00	1.28*	0.427*	ND	0.414*	0.927*	0.374*	ND	0.611*	1.91*	0.638*	0.458*	0.494*	10.2
Dacthal	ng/g	0.439-1.580	1.39-5.00	7.74	16.8	7.66	16.6	6.03	5.70	4.01	3.90	5.09	4.38	2.04*	1.77*	9.34
Dieldrin	ng/g	0.339-5.070	0.81-12.10	20.6	8.64	2.59	8.27	17.9	9.29	8.81	7.49	18.7	9.59	3.83	4.09	10.1
Endosulfan I	ng/g	0.751-2.700	2.78-10.00	ND	ND	ND	ND	1.89*	ND	ND	ND	ND	ND	ND	ND	19.1
Endosulfan II	ng/g	2.780-10.000	13.90-50.00	5.37*	ND	ND	ND	5.64*	ND	ND	ND	ND	ND	ND	ND	20.0
Endosulfan Sulfate	ng/g	2.780-10.000	13.90-50.00	4.79*	ND	ND	ND	4.45*	ND	ND	ND	ND	ND	ND	ND	19.7
Endrin	ng/g	0.653-2.350	2.78-10.00	1.60*	2.09*	5.70*	2.14*	1.54*	2.42*	2.14*	4.66*	1.56*	2.40*	1.65*	6.03	13.9
Hexachlorobenzene	ng/g	0.075-0.270	0.42-1.50	0.843	0.331*	ND	0.284*	0.778	0.306*	ND	ND	0.916	0.320*	ND	ND	8.86
Oxadiazon	ng/g	0.651-2.340	1.39-5.00	18.0	4.17	ND	3.68	16.9	4.17	3.10	3.61	19.4	4.36	1.73*	1.64*	32.6
Oxychlordane	ng/g	0.256-0.920	1.39-5.00	ND	ND	ND	ND	0.450*	ND	ND	ND	ND	ND	ND	ND	9.67
Parathion, Ethyl	ng/g	0.584-2.100	2.78-10.00	ND	ND	2.11*	ND	ND	ND	ND	ND	ND	1.34*	ND	1.54*	33.2
Total Chlordane	ng/g	0.270-2.450	1.39-5.00	19.77	4.370	ND	4.32	19.66	5.13	4.37	0.867	20.12	5.37	0.647	0.581	NA
Total DDT	ng/g	0.467-18.900	1.39-77.00	362.36	135.31	58.8	132.71	356.15	177.32	110.41	80.39	372.31	167.16	47.76	66.4	NA
Total DDT / OC	ug/g oc	NA	NA	11.54	5.71	3.09	4.74	14.84	7.39	3.94	2.45	14.43	10.07	1.83	2.29	NA
DBOB		Percent Surrogate Recovery		68.4	72.2	71.4	72.1	76.4	99.5	106	88.3	87.3	63.4	68.6	73.3	63.7
DDD*, p,p'		Percent Surrogate Recovery		72.1	81.4	80.9	80.5	80.7	109	106	95.9	85.8	71.6	73.7	75.9	73.8
DBCE		Percent Surrogate Recovery		88.5	82.5	76.4	86.7	89.9	84.4	95.1	85.2	74.7	86.6	82.6	99.5	80.1
Organophosphates																
Chlorpyrifos	ng/g	5.00	10.00	19.0	ND	ND	ND	15.3	ND	ND	ND	15.3	ND	ND	ND	78.6
Triphenyl phosphate		Percent Surrogate Recovery		124	111	102	100	115	110	109	111	109	114	111	115	89.2

* Detected not quantified

Table 7b. Summary of measured water concentrations of all chemicals detected in samples from the Tembladero Slough VTS.

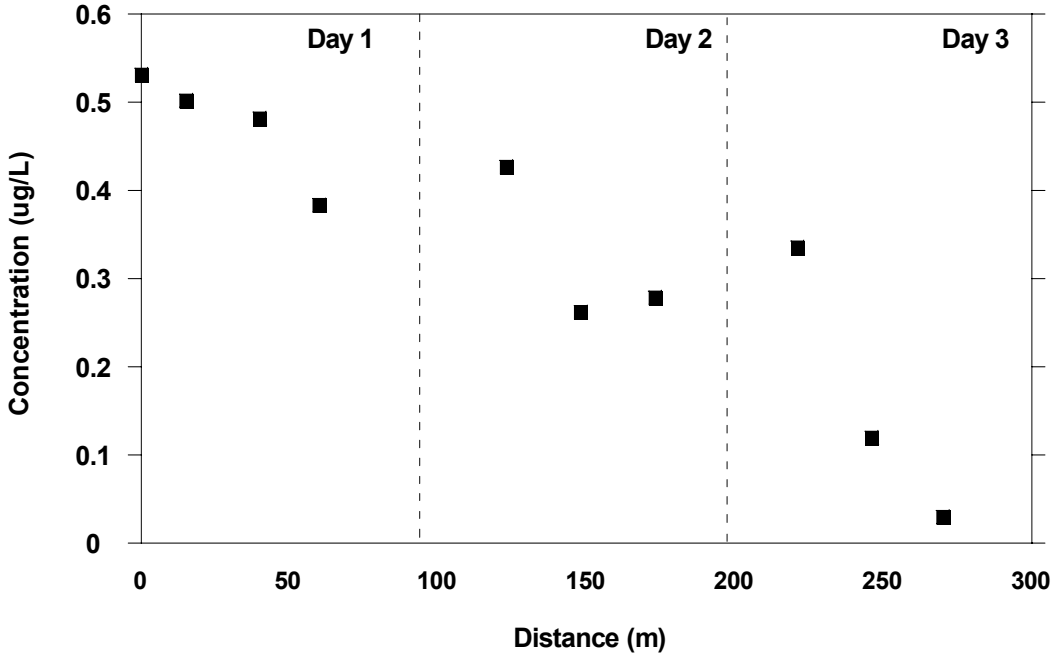
				August 23				September 20				November 1				
		MDL	RL	TEM-MOL	DCR-001	DCR-003	DCR-001D	TEM-MOL	DCR-001	DCR-003	DCR-004	TEM-MOL	DCR-001	DCR-003	DCR-004	LCS Recovery
Pyrethroids																
Cyfluthrin	ng/g	2.00	4.00	ND	ND	ND	ND	ND	ND	ND	3.25**	ND	ND	2.15**	ND	82.4
Cypermethrin	ng/g	2.00	4.00	4.17	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	68.6
(Es)Fenvalerate	ng/g	1.00	2.00	16.2	2.82	ND	2.42	11.5	3.30	2.32	ND	16.6	3.31	1.42**	ND	91.7
(Es)Fenvalerate / OC	ug/g oc	NA	NA	0.52	0.12	ND	0.08	0.41	0.14	0.08	ND	0.64	0.20	0.05	ND	NA
Fenpropathrin *	ng/g	2.00	4.00	4.98	ND	ND	ND	3.36**	ND	ND	ND	ND	ND	ND	ND	75.5
Lambda-cyhalothrin	ng/g	1.00	2.00	10.6	ND	ND	ND	5.44	ND	ND	ND	2.33	ND	ND	ND	64.2
Lambda-cyhalothrin / OC	ug/g oc	NA	NA	0.34	ND	ND	ND	0.19	ND	ND	ND	0.09	ND	ND	ND	NA
Permethrin	ng/g	4.00	8.00	33.1	ND	ND	ND	28.1	ND	ND	ND	30.5	ND	13.6	ND	80.1
Permethrin / OC	ug/g oc	NA	NA	1.05	ND	ND	ND	1.00	ND	ND	ND	1.18	ND	0.52	ND	NA
Dibromooctafluorobiphenyl	Percent Surrogate Recovery			89.4	85.2	93.1	99.3	99.0	125	136	115	99.9	83.1	94.7	96.9	98.1
Total Organic Carbon	%	0.01	0.03	3.14	2.37	1.90	3.05	2.80	2.40	2.80	3.28	2.58	1.66	2.61	2.90	NA
Grain Size Distribution																
Cobble	%	NA	NA	0	0	0	0	0	0	0	0	0	0	0	0	NA
Gravel	%	NA	NA	0	0	0	0	0	0	0	0	0	0	0	0	NA
Sand	%	NA	NA	3.78	2.2	3.33	3.63	15.4	5.9	5.67	5.31	2.78	8.04	4.53	5.6	NA
Fines	%	NA	NA	96.19	97.80	96.67	96.37	84.60	94.10	94.33	94.69	97.22	91.96	95.47	94.40	NA
Moisture	%	NA	NA	48.2	62.4	68.2	59.1	53.4	56.8	66.8	71.0	53.9	61.4	54.5	58.8	NA

** Below reporting limits

Measurements of a parcel of water through the treatment system

During one storm event, hydrologic model calculations were made to assist in tracking a parcel of water through the channelized portion of the Tembladero treatment system. A sample was collected from one location at the time the center point of the stormwater parcel was expected to be there, and one additional sample was collected approximately 25 m upstream, and another 25 m downstream to bracket the parcel. ELISA measurements of diazinon showed a steady decrease in the concentration of this pesticide as it moved through the channel (Figure 18). Diazinon entered the system at a concentration above the *C. dubia* LC50 (0.32 ug/L; Bailey et al. 1997). The concentration dropped to bracket the LC50 after one day, and averaged below the LC50 by the third day, as it exited the channel at station DCR-003.

Figure 18. Diazinon concentrations in samples collected from a parcel of water as it traveled down channel in the constructed wetland January 28, 29, and 30, 2007. The 0 meter mark is the inlet, the last sample is near the channel outlet, before water enters the broader shallow wetland. After the inlet sample, each daily group of three samples represent the expected upstream edge, center, and downstream edge of the parcel.



Toxicity data support the observed declines in diazinon concentration (Table 4). The samples at the inlet had 0% *C. dubia* survival, and that increased to 50% survival 60 m down the channel. Water in the channel was saltier than water being pumped in at the inlet, so parcel conductivity increased as the parcel was diluted with channel water. By the second day, parcel conductivity was high enough that samples had to be diluted in the laboratory to about 75% original strength in order to be tested within the *C. dubia* tolerance range. Even with dilution, there was only 20% survival at the upstream edge of the parcel (123 m), and this increased to 100% survival downstream (173 m). Samples collected on the third day had even higher conductivities, and had to be diluted to half strength for testing. *C. dubia* survival was 90 to 100% in these samples.

Table 8. Toxicity of a stormwater parcel tracked for three days through the Tembladero system channel. Input water was fresher than water in the channel, so conductivity increased as the pulse moved downstream. Toxicity tests were conducted on sample dilutions from days 2 and 3. Toxicity data were not normalized for dilution.

Date	Station (meters downstream from inlet)	Conductivity (uS/cm)	Diazinon Concentration (ug/L)	*C. dubia* Mean Survival (%)	Dilution (% strength)	*C. dubia* Mean Survival (%)
1/28/07	DCR-001	1611	0.532	0.00	na	na
1/28/07	15 m	1822	0.502	0.00	na	na
1/28/07	40 m	2110	0.482	0.10	na	na
1/28/07	60 m	2380	0.384	0.50	na	na
1/29/07	123 m	3330	0.427	0.00	75%	20%
1/29/07	148 m	3420	0.262	0.00	73%	70%
1/29/07	173 m	3370	0.279	0.00	74%	100%
1/30/07	221 m	4620	0.335	0.00	52%	90%
1/30/07	246 m	4770	0.120	0.00	51%	90%
1/30/07	DCR-003	4670	<RL	0.00	52%	100%

Diazinon reduction and dilution

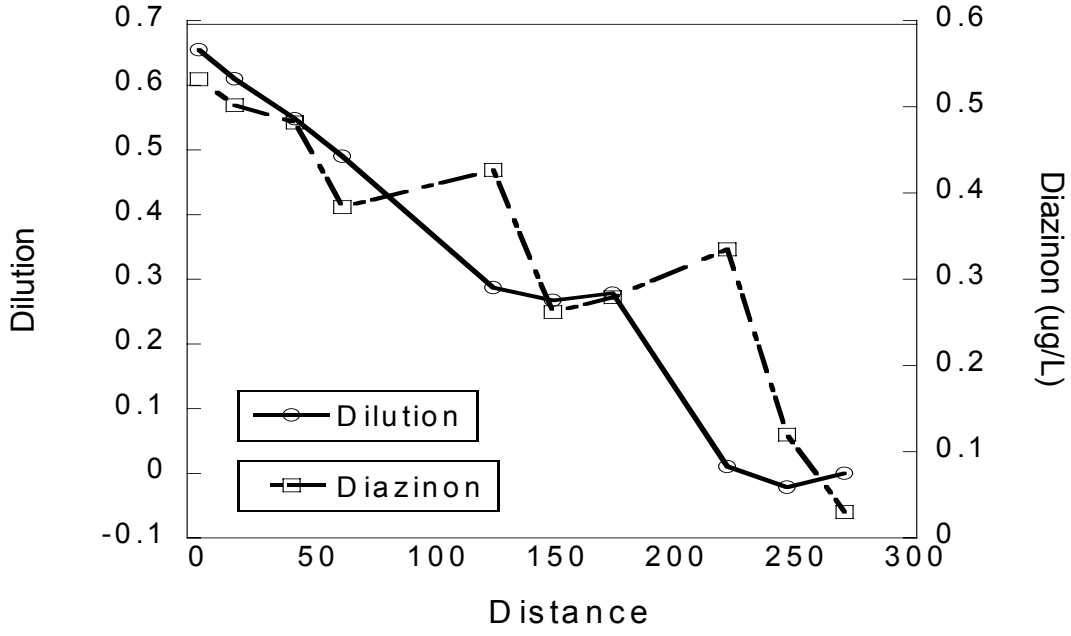
While diazinon concentrations decreased as the parcel of Slough water moved downstream through the constructed wetland, this was most likely due to dilution with channel water rather than retention or breakdown of this relatively soluble pesticide. This process can be evaluated by comparing diazinon concentrations to changes in salt content as the water parcel moved through

the system (Figure 19). Conductivity (salt content) measurements were not taken in the constructed wetland channel prior to pumping during this survey. However, conductivity was measured in all water samples collected during the survey, including the initial sample as Slough water entered the channel (time 0, distance 0), which had a conductivity of 1,611 uS/cm, and the final sample (day 3, distance 270 m, at the channel outflow), which had a conductivity of 4,670 uS/cm. This most-downstream measurement can be used as a baseline for channel water conductivity that existed prior to pumping water into the channel from Tembladero Slough. Given this assumption, the increase in conductivity of the water parcel as it passed through the channel can be used as a measure of parcel dilution. The left y-axis in Figure 19 represents parcel dilution (as proportional sample strength, the complement of dilution):

Dilution = 1 - (sample conductivity ÷ baseline conductivity).

The right y-axis is the diazinon concentration. The close alignment of the two measurements as the water parcel moved down-channel indicates that decreases in diazinon concentrations under these conditions were more likely due to dilution within the system than breakdown or

Figure 19. Diazinon concentrations in samples collected from a parcel of water as it traveled down-channel in the constructed wetland, matched with dilution of the water parcel with higher conductivity water that previously existing in the channel. See text for quantification of dilution values.



retention of the pesticide. As has been discussed earlier, diazinon breakdown is optimized in systems designed to increase water retention time, allow UV sunlight penetration for photolysis, and promote lower than neutral pH to enhance hydrolysis.

While the data suggest that dilution in the wetland system was most responsible for decreases in diazinon concentration, this is a useful finding. Pesticides tend to enter aquatic systems in pulses, following combinations of recent applications and either excessive irrigation or heavy precipitation. Wetland systems may serve to dampen peak concentrations before pulses enter aquatic systems of higher wildlife value.

Overall, this three-day study indicates the potential for pesticide reduction as water passes through a vegetated treatment system. Trends in some of the earlier surveys (surveys 1, 2, and 5) showed that downstream locations had lower pesticide concentrations and toxicity; but these trends provide strong evidence for treatment effectiveness only if it is assumed that input concentrations didn't change from the time water from the most downstream station entered the system. In other surveys, relatively high downstream concentrations could have been the result of higher input concentrations days before the survey. The data from the parcel tracking study support a conclusion that the constructed wetland was effective at reducing peak concentrations and toxicity of diazinon pulses passing through the system. Data for other pesticide compounds and classes indicate the potential for enhanced retention on plant surfaces or in sediments, with a concomitant potential for breakdown during these increased residence times.

Treatment system effectiveness as a function of residence time

The Tembladero constructed wetland was designed to have the capacity for changing flow rates to investigate treatment effectiveness as a function of residence time. This is an important consideration for future projects that might design new wetland areas to treat runoff from various size watersheds subject to different hydrologic conditions. In the current study, the organophosphates were the only class of chemicals detected frequently enough and at high enough concentrations to perform an analysis of treatment effectiveness in relation to flow.

When considering the total concentration of all measured organophosphates, and looking at the entire Tembladero system as the treatment unit, there appears to be little relationship

Figure 20. Relationship of channel flow rate (residence time) with reduction in concentrations of all detected organophosphate pesticides: in the total system, from channel inlet to wetland outlet. Pearson correlation coefficient = -0.624; n = 8; significance level: p = 0.10.

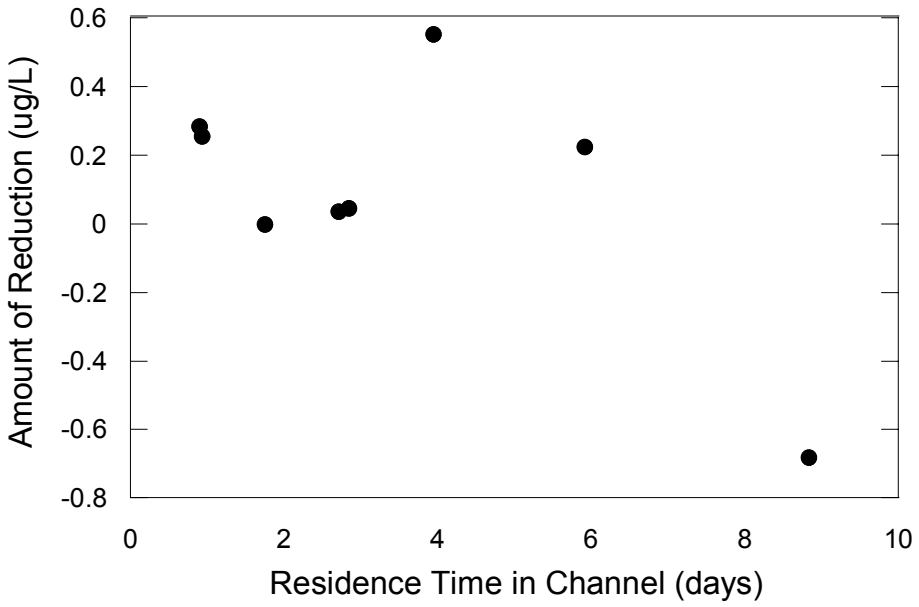


Figure 21. Relationship of channel flow rate (residence time) with reduction in concentrations of all detected organophosphate pesticides: in the channel, from channel inlet to channel outlet. Pearson correlation coefficient = -0.736; n = 8; significance level: p = 0.04.

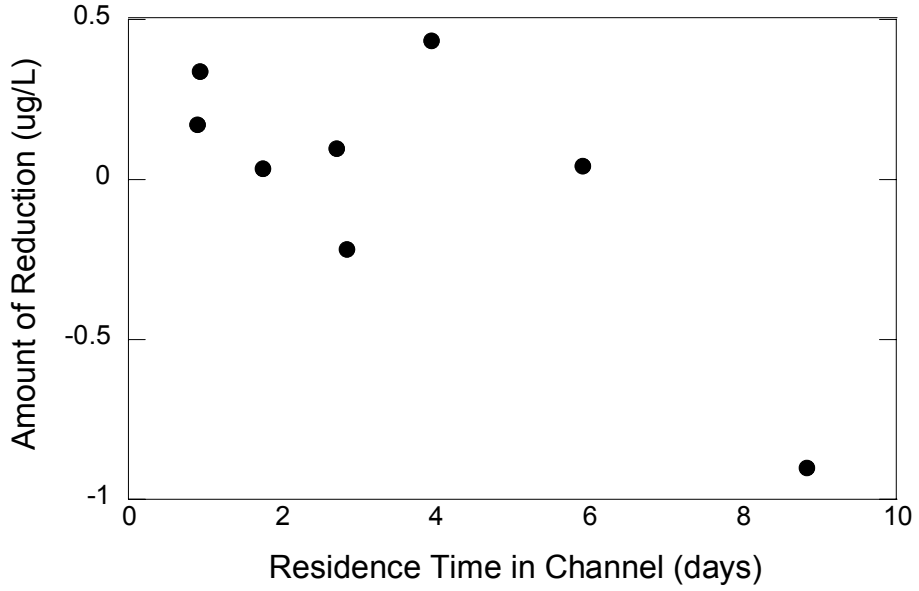
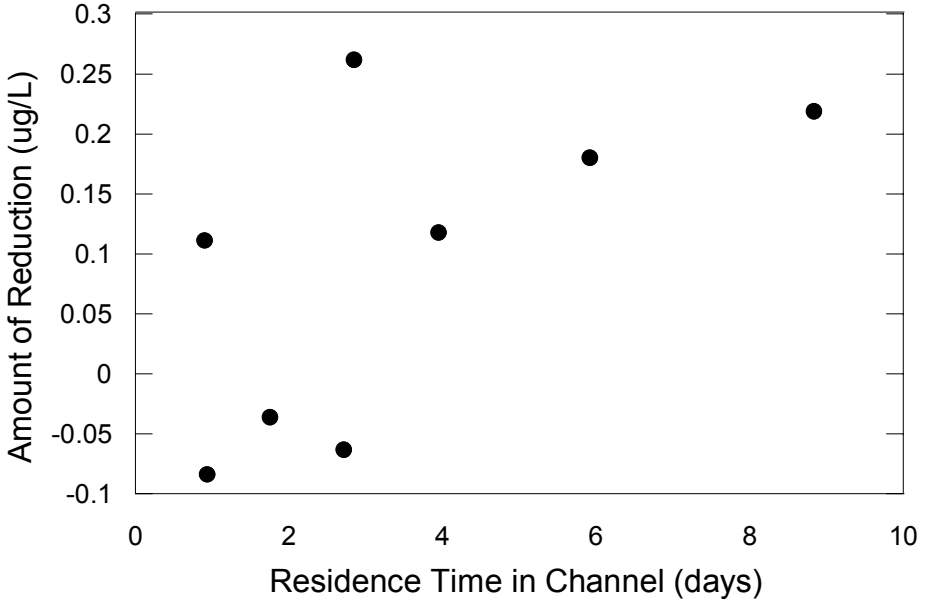


Figure 22. Relationship of channel flow rate (residence time) with reduction in concentrations of all detected organophosphate pesticides: in the wetland, from channel outlet to wetland outlet. Pearson correlation coefficient = 0.603; n = 8; significance level: p = 0.12.

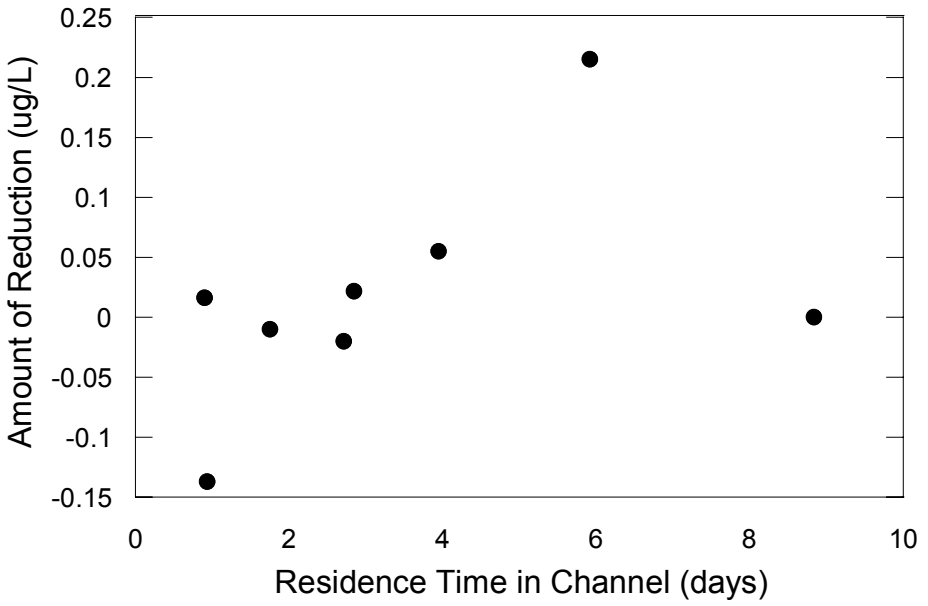


between concentration and retention time (Figure 19). In fact, the outlying point indicates less pesticide reduction with longer residence time in the system, which is somewhat counter intuitive, assuming that settlement, sorption, and breakdown processes take time to occur.

However, when the narrow channel and shallow wetland components of the system are considered separately, for total OPs, two different patterns emerge. In the channel component (Figure 20), pesticide reduction appears to be greatest with the lowest residence times. In the broad, shallow wetland component of the system, the trend is the reverse: longer residence times are associated with greater reductions in pesticide concentration (Figure 21).

This situation may be complicated, however, by consideration of all OPs together in terms of a single reduction in total concentration. Looking at the most frequently measured compound, diazinon, by itself, indicates a more consistent trend. In the whole treatment system, from the inlet at DCR-001 to the wetland outlet at DCR-004 (Figure 1), it appears that diazinon reduction increases with residence time, at least to a point (Figure 22). For diazinon,

Figure 23. Relationship of channel flow rate (residence time) with reduction in concentrations of diazinon: in the total system from channel inlet to wetland outlet. Pearson correlation coefficient = 0.470; n = 8; significance level: p = 0.24.



however, this trend holds for both the channel and the wetland, when each is considered separately (Figure 23 and 24). Given the caveats discussed in the introduction, care must be

Figure 24. Relationship of channel flow rate (residence time) with reduction in concentrations of diazinon: in the channel, from channel inlet to channel outlet. Pearson correlation coefficient = 0.365; n = 8; significance level: p = 0.38.

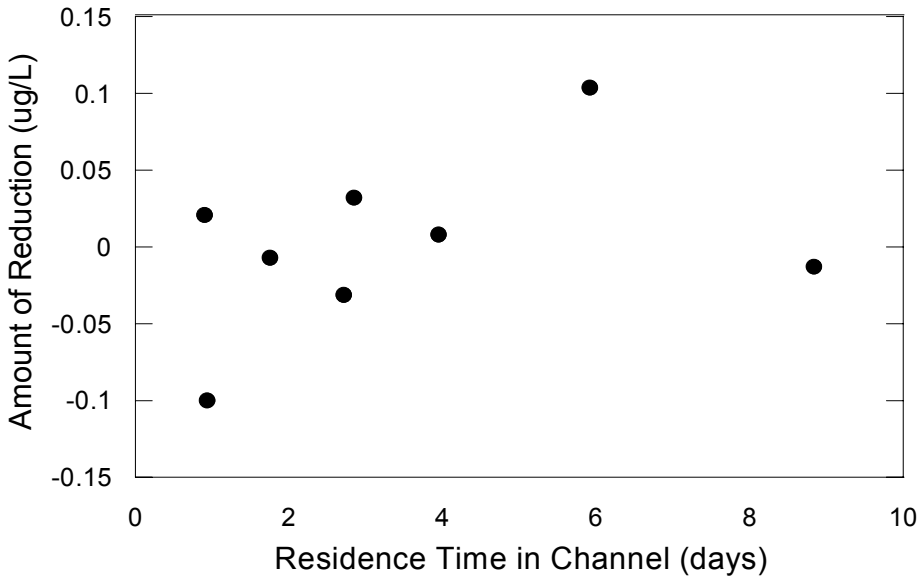
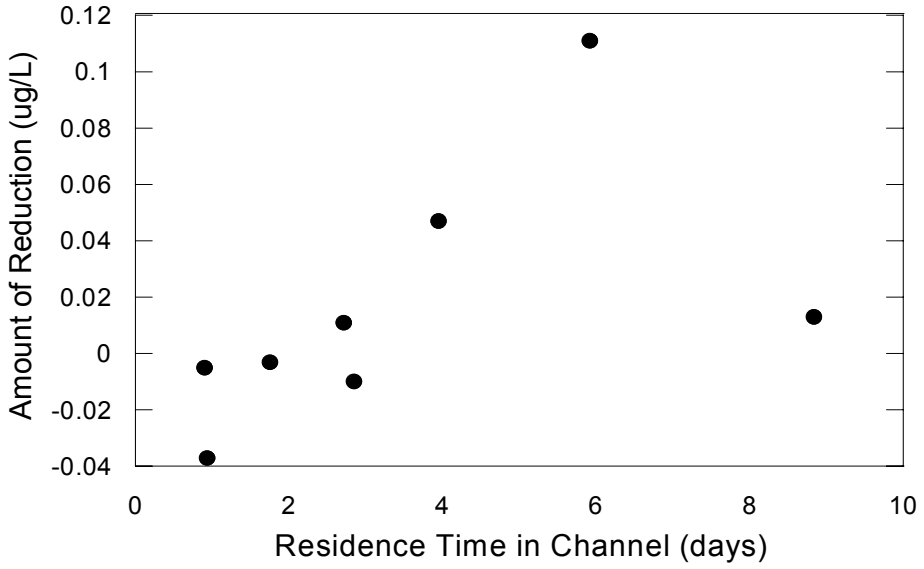


Figure 25. Relationship of channel flow rate (residence time) with reduction in concentrations of diazinon: in the wetland, from channel outlet to wetland outlet. Pearson correlation coefficient = 0.548; n = 8; significance level: p = 0.16.



taken to avoid drawing broad conclusions, but it does appear that longer residence times in the vegetated treatment system were associated with greater reduction rates for this pesticide. Considering potential breakdown pathways for diazinon, photolysis half-lives in water are on the order of days to weeks (Novartis, 1997). Hydrolysis half-lives are on the order of days at low pH (pH = 5), to weeks to months at neutral to higher pH (pH = 7 - 9). The pH of channel water during this study varied between 8 and 9. The channel was shallow enough (~ 0.5 m) to permit some light penetration to aid in photolysis, but the higher pH would indicate that hydrolysis rates would be slow relative to the study residence times. As discussed in a subsequent section, there was no apparent relationship between diazinon concentration and suspended particle concentration. So the greater diazinon reduction with longer residence time may be the result of increased sorption to plant materials, with some additional breakdown by photolysis, and less removal associated with hydrolysis or settlement of suspended particles.

On-farm vegetated treatment systems

Vegetated treatment system pond at site G-09

As described above, the VTS system at site G-09 was a single pond with multiple inlets. Samples were collected from the inflow at the main inlet at the upstream end of the pond (station A), and from the single outlet at the downstream end (station B).

In four of five surveys, all water samples collected from both stations were highly toxic to *C. dubia* (Table 9). In one survey, survival was 80% at both stations. Sediment samples collected at both stations were also highly toxic, producing 0% amphipod survival (Table 9).

Table 9. Toxicity, ELISA, turbidity, and nutrient summary for site G-09 surveys.

			Ceriodaphnia				Chlor.	Diaz.	Turbidity	Nitrate	Phosphate	Hyalella Sediment			
No.	Date	Station	Mean % Survival	Mean # Neonates	SD # Neonates	LT50	ng/L	ng/L	NTU	mg/L	mg/L	Mean % Survival	SD % Survival	Mean Growth (mg/ind)	SD Growth (mg/ind)
1	7/7/06	G-09 A	40*	13	6.7	5.8	336	<RL	18.3	34.0	4.93				
		G-09 B	0*	5	3.9	4.4	281	<RL	3.4	45.2	8.73				
2	7/26/06	G-09 A	80	16	7.7	NA	277	ND	4.8	38.4	6.84				
		G-09 B	80	26	3.7	NA	245	ND	5.6	50.6	10.48				
3	8/23/06	G-09 A	0*	2*	7.7	1.9	234	ND	7.4	22.6	6.9				
		G-09 B	0*	10*	3.7	4.5	147	ND	1.6	18.9	6.3				
4	10/2/06	G-09 A*	0*	0*	0.0	0.5	224	0.129	157	51.2	26.2				
		G-09 B	0*	1*	1.1	2.8	171	<RL	1.5	4.2	23.5				
5	10/27/06	G-09 A**	0*	0*	0.0	0.5	461	<RL	13.8	56.4	17.9	0*	0	NA	NA
		G-09 B	0*	0*	0.0	2.5	330	<RL	3.7	42.8	14.3	0*	0	NA	NA

* Significantly toxic: statistically significant difference from the control and endpoint less than the toxicity threshold.

Toxicity identification in site G-09 samples

Toxicity identification evaluations (TIEs) were conducted to identify chemicals of concern. Agricultural runoff frequently contains numerous constituents, and not all occur at concentrations capable of causing adverse effects to aquatic ecosystems. The TIEs were designed to identify those chemicals causing toxicity to aquatic invertebrates, so that the vegetated treatment systems could be evaluated for effectiveness in reducing concentrations of these chemicals. VTS effectiveness in reducing nutrient concentrations and turbidity are considered in a later section. TIEs were conducted on a water sample collected October 2. Solid-phase and interstitial water TIEs were conducted on a sediment sample collected October 27.

Complete mortality was observed in the water sample from station G-09 A (inlet) collected October 2. The concentration of chlorpyrifos was 224 ng/L, or approximately four times the LC50 of *C. dubia* (0.053 μg/L; Bailey et al., 1997). Toxicity was reduced with the centrifugation treatment, which is a pre-treatment for the solid-phase extraction columns, but the columns further reduced toxicity (Table 10). The cation column completely removed toxicity and chlorpyrifos, but the eluate of this column did not return toxicity or any organophosphate. The HLB column also completely removed toxicity and chlorpyrifos, but the eluate of this column returned some toxicity and a detectable amount of chlorpyrifos. The sequential column treatment completely removed toxicity, but only the cation eluate returned toxicity. It is suspected that this result is erroneous because at 96 hours there was significant blank toxicity in the cation eluate samples. Carboxylesterase reduced approximately the same amount of toxicity as the centrifugation treatment, but the BSA also reduced toxicity, indicating that pyrethroids were probably not implicated. Piperonyl butoxide addition reduces the toxicity of organophosphate pesticides, but significant blank toxicity was noted in the PBO treatments, thus making the PBO data suspect in this case. The reduction of toxicity and chlorpyrifos concentration by the HLB column, and the return of toxicity and a measurable amount of chlorpyrifos indicate that this organophosphate was probably the main cause of toxicity. Subsequent chemical analysis found that the chlorpyrifos concentration in this sample was 0.762 ug/L (Table 13), about 14 times the LC50 for *C.*

dubia (Bailey et al. 1997). In fact, the chlorpyrifos LC50 was exceeded in all G-09 water samples (Table 13).

Table 10. Results of toxicity identification evaluation of site G-09 Oct 2 water sample.

G-09 A	Percent Sample											
	0%		10%		25%		50%		100%		Toxic	Chlorpyrifos
Treatment	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Units	ng/L
Baseline	100	0	89	19	44	38	0	0	0	0	4.7	238
Centrifuge	100	0	89	19	100	0	0	0	0	0	2.9	168
Cation Column	100	0	100	0	100	0	89	19	89	19	<1	ND
Cation Eluate	44	38	67	33	67	33	11	19	22	19	NA	ND
HLB Column	78	19	89	19	0	0	89	19	100	0	<1	ND
HLB Eluate	100	0	100	0	89	19	89	19	0	0	1.7	<RL
Sequential Columns	100	0	100	0	100	0	100	0	100	0	<1	ND
Sequential Cation Eluate	11	19	0	0	0	0	0	0	0	0	NA	ND
Sequential HLB Eluate	100	0	100	0	100	0	100	0	100	0	<1	ND
Carboxylesterase	100	0	89	19	89	19	0	0	0	0	2.9	235
BSA	100	0	100	0	100	0	0	0	0	0	2.8	233
PBO	0	0	0	0	0	0	0	0	0	0	NA	227
Carboxylesterase/PBO	11	19	0	0	0	0	0	0	0	0	NA	NA

Two TIEs were conducted on sediment from the G-09 inlet (station A). The first TIE incorporated solid-phase treatments, while the second TIE subjected the interstitial water to standard water treatments. Both types of TIEs utilized the amphipod *H. azteca*. None of the solid-phase treatments reduced toxicity (Table 11), but two amendments were eluted to determine if a toxicity signal could be returned to clean dilution water. The Ambersorb eluate was highly toxic, and the SIR-300 was non-toxic, indicating toxicity due to an organic chemical. Sediment chemical analysis later revealed that the sediment concentration of chlorpyrifos was 11,258 ng/g (Table 14a), about 30 times greater than the published *H. azteca* LC50 of 399 ng/g (Brown et al. 1997). This extremely high concentration helps explain why sample toxicity could not be reduced by any of the solid-phase TIE manipulations, even though toxicity could be returned through elution of the Ambersorb resin beads.

Table 11. Results of a toxicity identification evaluation of site G-09 sediment sample. Values are % survival of test amphipods.

	Solid-Phase TIE		Amendment Elution	
Treatment	Mean	SD	Mean	SD
G-09 A	0	0		
G-09 A (10% Ambersorb)	0	0	0	0
Control (10% Ambersorb)	98	4	73	31
G-09 A (10% PCC)	0	0		
Control (10% PCC)	74	11		
G-09 A (10% SIR-300)	0	0	100	0
Control (10% SIR-300)	94	9	93	12
G-09 A (Enzyme)	0	0		
Control (Enzyme)	96	9		
G-09 A (BSA)	0	0		
Control (BSA)	97	4		
G-09 A (10% Control)	0	0		
Sediment Control	96	9		
Ambersorb Elution Control			93	12
SIR-300 Elution Control			93	12

Interstitial water extracted from the G-09 sediment was significantly toxic (18.6 toxic units). In a TIE, a toxic unit (TU) = 100 divided by the sample LC50, determined by testing a dilution series of the sample. In this case, half the amphipods died at a sample concentration of just over 5% (Table 12). ELISA measurement found a sample concentration of 839 ng/L chlorpyrifos. Passing the sample through a cation column significantly reduced the chlorpyrifos concentration to 105 ng/L, but only reduced the toxicity to 14.7 TU. The cation eluate did not return toxicity or chlorpyrifos to clean dilution water. The HLB column completely removed the chlorpyrifos but only reduced the toxicity to 16.4 TUs, but the eluate returned toxicity and 95% of the chlorpyrifos. The sequential columns removed the toxicity, but only the HLB eluate returned toxicity to clean dilution water. It was assumed that most of the chlorpyrifos was adsorbed onto the cation column, but was not eluted with the acid fraction. The addition of the enzyme reduced toxicity to 6 TUs, but did not reduce the chlorpyrifos concentration. This result indicates the presence of a pyrethroid pesticide. The addition of BSA (a control for the enzyme treatment) did not reduce toxicity, which also supports toxicity caused by a pyrethroid. The addition of PBO individually and in combination with the enzyme increased toxicity slightly, but because the lowest concentration tested was 10%, the

calculated TU value cannot exceed 20, therefore it is difficult to determine if toxicity was significantly increased. Based on the above results (Table 12), the probable cause of toxicity is a combination of chlorpyrifos and a pyrethroid. Interstitial water chemical measurements were not available, but the solid phase chemistry for this sample indicated high concentrations of both chlorpyrifos and the pyrethroid pesticide permethrin (Table 14), and the evidence implicates these two chemicals as the toxic agents in the G-09 sediment.

Table 12. Results of toxicity identification evaluation of site G-09 porewater sample.

G-09 A	Percent Sample											
	0%		10%		25%		50%		100%		Toxic	Chlorpyrifos
Treatment	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Units	ng/L
Baseline	93	12	07	12	0	0	0	0	0	0	18.6	839
Cation Column	100	0	27	12	07	12	0	0	0	0	14.7	105
Cation Eluate	87	12	100	0	100	0	100	0	93	12	<1	<RL
HLB Column	87	12	0	0	20	20	27	23	13	12	16.4	<RL
HLB Eluate	93	12	20	20	0	0	0	0	0	0	15.7	793
Sequential Columns	100	0	28	24	0	0	0	0	0	0	14.5	<RL
Sequential Cation Eluate	93	12	100	0	100	0	100	0	100	0	<1	<RL
Sequential HLB Eluate	87	12	87	23	87	12	0	0	0	0	2.8	123
Carboxylesterase	100	0	87	12	20	20	0	0	0	0	6.0	806
BSA	93	12	0	0	0	0	0	0	0	0	20.0	787
PBO	100	0	0	0	0	0	0	0	0	0	20.0	960
Carboxylesterase/PBO	93	12	0	0	0	0	0	0	0	0	20.0	887

Table 13. Summary of measured water concentrations of all chemicals detected in samples from the G-09 VTS.

				7/7/06		7/26/06		8/23/06		10/2/06		10/27/06			
Organophosphates		**MDL**	**RL**	**G-09 A**	**G-09 B**	**G-09 A**	**G-09 B**	**G-09 A**	**G-09 B**	**G-09 A**	**G-09 B**	**G-09 A**	**G-09 A Dup**	**G-09 B**	**LCS Recovery**
Chlorpyrifos	ug/L	0.003	0.005	0.152	0.125	0.218	0.157	0.280	0.120	0.762	0.158	0.436	0.452	0.090	83.2-101
Diazinon	ug/L	0.005	0.020	0.023	0.038	0.032	0.036	0.036	0.040	0.041	0.025	0.026	0.029	0.027	83.8-112
Dioxathion	ug/L	0.030	0.050	ND	ND	ND	ND	1.13	0.540	1.74	2.40	0.570	0.456	0.337	87.6-106
Thionazin	ug/L	0.020	0.050	ND	ND	ND	0.111	ND	ND	ND	ND	ND	ND	ND	88.6-113
Triphenyl phosphate	Percent Surrogate Recovery			92.1	104	109	106	103	115	111	108	109	105	92.8	NA
Pyrethroids															
Bifenthin	ug/L	0.001	0.002	ND	ND	ND	ND	ND	ND	0.007	ND	ND	ND	ND	70.4-103
Cyfluthrin	ug/L	0.002	0.004	ND	ND	ND	ND	ND	ND	ND	ND	0.040	0.039	0.026	79.6-103
Cypermethrin	ug/L	0.002	0.004	ND	ND	ND	ND	ND	ND	ND	ND	0.020	0.019	0.011	73.3-104
Permethrin	ug/L	0.003	0.005	0.151	ND	ND	ND	ND	ND	0.998	0.003	ND	ND	ND	78.4-106
Dibromooctafluorobiphenyl	Percent Surrogate Recovery			94.5	107	90	82.2	104	109	102	116	88.4	94.5	91.7	NA
Organochlorines															
DDE(p,p')	ug/L	0.001	0.002	ND	ND	ND	ND	ND	ND	0.008	ND	ND	ND	ND	79.3-112
DDT(o,p')	ug/L	0.001	0.002	0.004	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	72.9-117
Endosulfan I	ug/L	0.001	0.002	0.021	0.009	ND	ND	ND	ND	ND	ND	ND	ND	ND	80.8-104
Endosulfan II	ug/L	0.001	0.002	0.023	ND	ND	ND	ND	ND	0.010	0.005	ND	ND	ND	80.4-109
Endosulfan sulfate	ug/L	0.001	0.002	0.042	ND	ND	ND	ND	ND	0.016	0.014	ND	ND	ND	17.7-108
Oxadiazon	ug/L	0.001	0.002	ND	ND	ND	ND	ND	ND	0.021	0.012	ND	ND	ND	83.7-109
Dibromooctafluorobiphenyl	Percent Surrogate Recovery			94.5	107	113	92	124	112	111	103	84.5	87.3	102	NA
Carbamates															
Methomyl	ug/L	0.010	0.020	ND	ND	ND	ND	ND	0.150	ND	ND	0.073	ND	ND	82.9-105

Table 14a. Summary of measured sediment concentrations of all chemicals detected in samples from G-09 and SV-03 (11/22/2007).

		MDL	RL	G-09 A	G-09 B	SV-03 A	SV-03 C	LCS Recovery
Organochlorines								
Aldrin	ng/g	0.181-0.650	1.39-5.00	ND	ND	0.216*	2.05	102
Chlordene, Alpha	ng/g	0.192-0.690	0.70-2.50	ND	ND	0.564*	1.14	99.00
Dacthal	ng/g	0.439-1.580	1.39-5.00	ND	2.56	4.85	6.15	93.4
Dieldrin	ng/g	0.339-5.070	0.81-12.10	23.7	25.6**	20.1**	15.3	101
Endosulfan I	ng/g	0.751-2.700	2.78-10.00	4.96*	1.96*	ND	ND	95.5
Endosulfan II	ng/g	2.780-10.000	13.90-50.00	16.5*	7.46*	ND	ND	100.0
Endosulfan Sulfate	ng/g	2.780-10.000	13.90-50.00	19.1*	13.1*	ND	ND	98.5
Endrin	ng/g	0.653-2.350	2.78-10.00	5.78*	4.12*	6.64	2.61*	139
Hexachlorobenzene	ng/g	0.075-0.270	0.42-1.50	1.14	1.52	0.415*	0.384*	88.6
Oxadiazon	ng/g	0.651-2.340	1.39-5.00	11.2	6.20	ND	ND	109
Total Chlordane	ng/g	0.270-2.450	1.39-5.00	1.71	ND	20.87	21.61	NA
Total DDT	ng/g	0.467-18.900	1.39-77.00	304.04**	505.09**	546.2	470.45	NA
Total DDT / OC	ug/g oc	NA	NA	4.37	20.37	23.05	22.73	NA
DBOB	ng/g	Percent Surrogate Recovery		67.0	70.8	68.3	75.3	63.7
DDD*, p,p'	ng/g	Percent Surrogate Recovery		82.0	93.3	100	85.2	73.8
DBCE	ng/g	Percent Surrogate Recovery		78.5	93.1	100	84.4	80.1
Organophosphates								
Chlorpyrifos	ng/g	5.00	10.00	11258**	2199**	ND	ND	78.6
Diazinon	ng/g	5.00	10.00	128	57.2	ND	ND	90.4
Triphenyl phosphate	ng/g	Percent Surrogate Recovery		104	117	109	107	89.2

* Detected not quantified

** Analytes analyzed at a secondary dilution

Table 14b. Summary of measured sediment concentrations of all chemicals detected in samples from G-09 and SV-03 (11/22/2007).

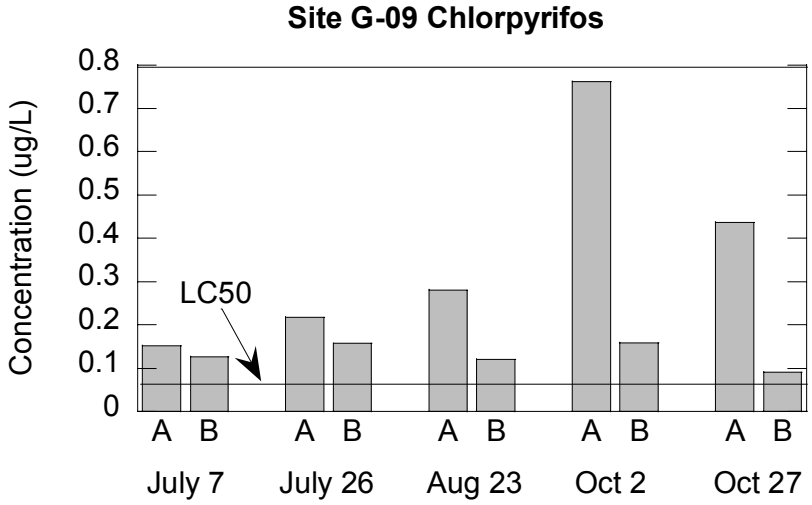
		MDL	RL	G-09 A	G-09 B	SV-03 A	SV-03 C	LCS Recovery
Pyrethroids								
Cypermethrin	ng/g	2.00	4.00	ND	ND	66.4**	ND	68.6
(Es)Fenvalerate	ng/g	1.00	2.00	ND	ND	14.7	17.4	91.7
(Es)Fenvalerate / OC	ug/g oc	NA	NA	ND	ND	0.62	0.84	NA
Lambda-cyhalothrin	ng/g	1.00	2.00	ND	ND	18.8	5.75	64.2
Lambda-cyhalothrin / OC	ug/g oc	NA	NA	ND	ND	0.79	0.28	NA
Permethrin	ng/g	4.00	8.00	2003**	412**	ND	ND	80.1
Permethrin / OC	ug/g oc	NA	NA	28.78	16.61	ND	ND	NA
Dibromooctafluorobiphenyl	ng/g	Percent Surrogate Recovery		131	92.7	98.2	99.4	98.1
Total Organic Carbon	%	0.01	0.03	6.96	2.48	2.37	2.07	NA
Grain Size Distribution								
Cobble	%	NA	NA	0	0	0	0	NA
Gravel	%	NA	NA	0	0	0	0.93	NA
Sand	%	NA	NA	33.28	47.85	7.69	10.44	NA
Fines	%	NA	NA	66.72	52.15	92.31	88.63	NA
Moisture	%	NA	NA	66.5	54.6	28.2	38.4	NA

** Analytes analyzed at a secondary dilution

Pesticide reduction at site G-09

While toxic concentrations of chlorpyrifos were present in all G-09 water samples, there was a significant decline in this pesticide from inlet to outlet in all five surveys (Figure 25). Reduction was likely the result of a number of processes, including bacterial breakdown on plant surfaces and particle binding and settlement (discussed below). Residence times in the pond are likely far less than the minimum of 16 to 30 days necessary for such reductions to occur via hydrolysis or photolysis.

Figure 26. Chlorpyrifos concentrations at the inlet (A) and outlet (B) of the VTS at site G-09**.**



Diazinon concentrations were measured in all samples at about an order of magnitude lower than their LC50 concentration. Diazinon is more water soluble than chlorpyrifos, and does not have as strong an affinity for particle binding. There was no indication from the data that diazinon concentrations were reduced in the G-09 VTS (Figure 26). This result, however, must be considered in the context of sample collection timing, as discussed in the Introduction.

Dioxathion was detected in water samples during three of five surveys at G-09. There was no consistent trend in concentration between inlet and outlet stations (Figure 27).

Figure 27. Diazinon concentrations at the inlet (A) and outlet (B) of the VTS at site G-09.

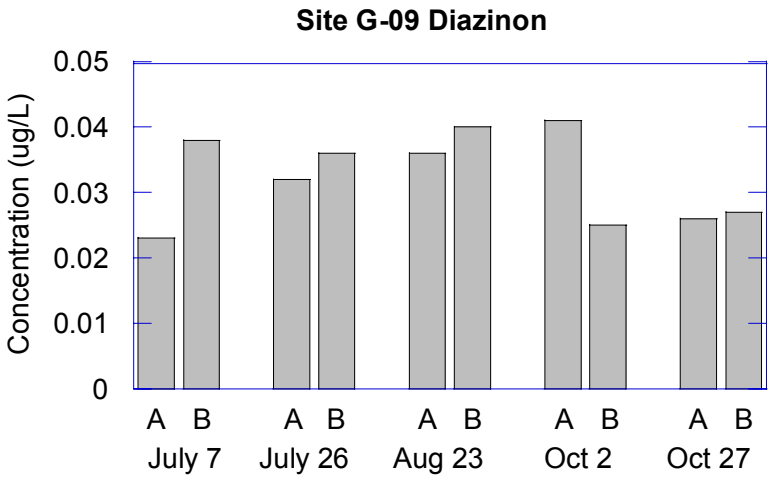
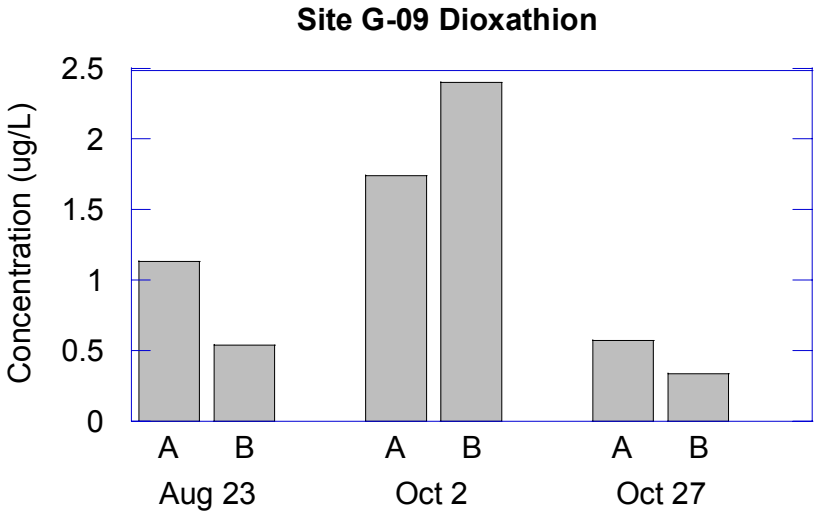


Figure 28. Dioxathion concentrations at the inlet (A) and outlet (B) of the VTS at site G-09.



Given the constraints of the sampling design (see Introduction), a useful way of evaluating pond effectiveness is to look at all chemicals that were detected at the inflow during all surveys, and calculate the mean difference in their concentrations at stations A and B (Figure 28). Diazinon averaged about 10% higher at the outlet than at the inlet (Figures 26 and 28). This is likely an artifact of sample timing and an inability to predict parcel transport through a vegetated system governed by pulse inputs. All other pesticide compounds or

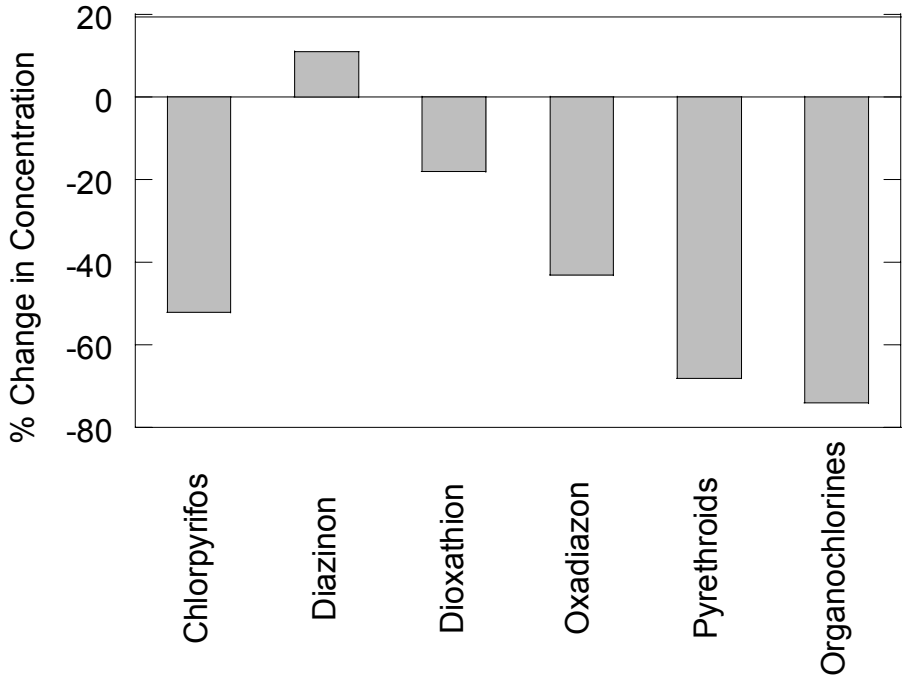
classes had lower average concentrations at the outlet (station B) than at the inlet (station A). Chlorpyrifos, diazinon, dioxathion, and oxadiazon were the most frequently measured OP pesticides in the system. Pyrethroids and organochlorines were generally present in very low concentrations in water, so they were summed into class totals for this analysis (Figure 28).

As with the Tembladero system, paired-sample t-tests were used to test the null hypothesis that pond inlet and outlet concentrations were the same. Statistically significant reductions were found for chlorpyrifos ($p = 0.047$) and organochlorines ($p = 0.017$). The null hypothesis could not be rejected for the following chemical categories: all chemicals combined, total OPs, or diazinon. Carbamates and pyrethroids had too few detections to allow statistical inference.

Sediment chemistry

There were differences in sediment concentrations of a number of chemicals measured during a sediment survey in which samples were collected at the G-09 VTS inlet and outlet. Since chlorpyrifos was implicated as a cause of toxicity in water and sediments from this system, it's important to note that sediment chlorpyrifos concentrations declined from 11,258 ng/g at the inlet to 2,199 ng/g at the outlet. Sediment concentrations of diazinon also declined from 128 ng/g to 57.2 ng/g. Total organic carbon, which tends to bind and be associated with sediment organic contaminants, was measured at 6.96% in inlet sediment and 2.48% in outlet sediment. Permethrin, which was also implicated in sediment toxicity, also declined steeply from 2,003 ng/g at the inlet to 412 ng/g at the outlet. No other pyrethroid pesticides were detected. Most organochlorine pesticides had lower concentrations in the outlet sediment, but DDT was higher (505 ng/g outlet, 304 ng/g inlet). This may reflect typical variability in sediment concentrations of this compound, which is fairly ubiquitous in soils that have been farmed for many years. In general, it appears that most sediment-associated pesticides, including those responsible for toxicity, were decreasing in concentration, most likely due to particle binding and settlement in the VTS system.

Figure 29. Change in concentration for all chemicals detected in water at both the inflow and outflow of the VTS at G-09. Values are means for all surveys in which chemicals were detected. Methomyl was detected at the outflow but not in the inflow from the same survey.



Vegetated treatment system at site SV-03

In all five surveys of the VTS at SV-03, samples from all three stations were highly toxic to *C. dubia* (0% survival), with the exception of 80% survival at the inlet station in the last survey (Table 15). Sediment toxicity was also high in the one sediment survey, with 0% amphipod survival at the inlet, and 28% survival at the outlet (Table 15).

Table 15. Toxicity, ELISA, turbidity, and nutrient summary for site SV-03 surveys.

			Ceriodaphnia				Chlor.	Diaz.	Turbidity	Nitrate	Phosphate	Hyalella Sediment			
No.	Date	Station	Mean % Survival	Mean # Neonates	SD # Neonates	LT50	ng/L	ng/L	NTU	mg/L	mg/L	Mean % Survival	SD % Survival	Mean Growth (mg/ind)	SD Growth (mg/ind)
1	7/11/06	SV-03 A	0*	0*	0.3	1.5	<RL	351	>1000	25.6	8.61				
		SV-03 B	0*	0*	0.0	0.5	ND	722	21.3	16.4	3.63				
		SV-03 C	0*	0*	0.0	0.5	ND	690	6.5	18.3	3.35				
2	7/26/06	SV-03 A	0*	1*	1.4	4.0	<RL	360	>1000	24.8	95.9				
		SV-03 B	0*	1*	1.8	2.9	<RL	617	38.5	17.4	5.58				
		SV-03 C	0*	1*	1.1	3.1	<RL	959	10.4	16.9	4.00				
3	8/23/06	SV-03 A	0*	1*	1.4	1.4	ND	438	>1000	32.4	73.6				
		SV-03 B	0*	1*	1.8	1.4	ND	582	403	19.7	20.9				
		SV-03 C	0*	1*	1.1	1.4	ND	328	159	20.4	11.9				
4	10/4/06	SV-03 A*	0*	0*	0.0	0.7	ND	11135	>1000	54.4	236				
		SV-03 B	0*	0*	0.0	1.0	ND	8280	310	42.2	19				
		SV-03 C	0*	0*	0.0	0.7	ND	3520	6.2	23.4	3.6				
5	10/27/06	SV-03 A	80	7*	3.8	NA	ND	<RL	>1000	30.6	48	0*	0	NA	NA
		SV-03 B	0*	5*	2.6	4.9	ND	189	144	22.7	9.6				
		SV-03 C	0*	0*	0.0	1.2	ND	380	18.4	25.5	3.4	28*	28	0.091	0.083

* Significantly toxic: statistically significant difference from the control and endpoint less than the toxicity threshold.

Toxicity identification in site SV-03 samples

Complete mortality was observed in the October 4 input water sample from SV-03. The baseline sample was toxic at 10% strength (>20 TU; Table 16), and ELISA measurements indicated 11,135 ng/L diazinon (Table 16), or approximately 35 diazinon TUs. (For individual chemicals, TU = the sample concentration divided by the chemical LC50, which is 320 ng/L for diazinon). The centrifuge treatment removed some of the diazinon, but none of the toxicity. Passing the sample through the HLB column removed all of the toxicity and all of the diazinon. The HLB eluate returned the toxicity and 83% of the diazinon. The carboxylesterase enzyme did not reduce toxicity, nor did the addition of BSA, indicating that any contribution by pyrethroids was overwhelmed by other chemicals. The addition of PBO individually and with carboxylesterase reduced toxicity to 2.7 and 2.9 TUs, respectively, indicating toxicity caused by an organophosphate pesticide. Subsequent GCMS chemical analysis confirmed high diazinon concentration (9,620 ng/L), along with high concentrations of the OP pesticide dimethoate (Table 17).

Table 16. Results of toxicity identification evaluation of site SV-03 water sample.

SV-03 A	Percent Sample											
	0%		10%		25%		50%		100%		Toxic	Diazinon
Treatment	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Units	ng/L
Baseline	100	0	0	0	0	0	0	0	0	0	20	11135
Centrifuge	100	0	0	0	0	0	0	0	0	0	20	9523
HLB Column	100	0	100	0	100	0	100	0	100	0	<1	ND
HLB Eluate	100	0	0	0	0	0	0	0	0	0	20	7932
Carboxylesterase	100	0	0	0	0	0	0	0	0	0	20	10241
BSA	100	0	0	0	0	0	0	0	0	0	20	9908
PBO	100	0	80	0	80	0	27	12	0	0	2.7	10218
Carboxylesterase/PBO	100	0	100	0	87	12	13	12	0	0	2.9	10469

Table 17. Summary of measured water concentrations of all chemicals detected in samples from the SV-03 VTS.

				7/10/2007		7/26/2007		8/23/2007		10/5/2007		10/30/2007			
Organophosphates		**MDL**	**RL**	**SV-03 A**	**SV-03 C**	**SV-03 A**	**SV-03 C**	**SV-03 A**	**SV-03 C**	**SV-03 A**	**SV-03 C**	**SV-03 A**	**SV-03 AD**	**SV-03 C**	**LCS Recovery**
Chlorpyrifos	ug/L	0.003	0.005	ND	0.021	ND	ND	ND	ND	ND	ND	ND	ND	ND	83.2-101
Diazinon	ug/L	0.005	0.020	0.280	0.548	0.230	0.404	0.230	0.208	9.62	2.46	0.020	0.025	0.305	83.8-112
Dimethoate	ug/L	0.030	0.050	ND	ND	1.39	1.31	0.564	0.960	8.40	3.24	0.806	ND	ND	16.7-107
Ethion	ug/L	0.020	0.050	ND	ND	ND	ND	ND	ND	0.052	0.02	ND	ND	ND	86.3-113
Malathion	ug/L	0.030	0.050	ND	ND	0.064	0.036	ND	ND	ND	ND	ND	ND	ND	54.7-104
Thionazin	ug/L	0.020	0.050	ND	ND	0.184	ND	ND	ND	ND	ND	ND	ND	ND	88.6-113
Triphenyl phosphate	Percent Surrogate Recovery			91.4	100	105	99.3	110	103	100	101	97.1	108	113	NA
Pyrethroids															
Cyfluthrin	ug/L	0.002	0.004	ND	ND	ND	ND	ND	ND	ND	ND	0.013	0.017	0.013	79.6-103
Cypermethrin	ug/L	0.002	0.004	ND	ND	0.608	ND	ND	ND	ND	ND	0.040	0.037	0.014	73.3-104
L-Cyhalothrin	ug/L	0.001	0.002	ND	0.002	ND	ND	ND	ND	ND	ND	ND	ND	ND	78.8-103
Permethrin	ug/L	0.003	0.005	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.033	78.4-106
Dibromooctafluorobiphenyl	Percent Surrogate Recovery			71.4	91.8	81.9	96.4	91.5	101	82.5	91.4	106	112	111	NA
Organochlorines															
Dacthal	ug/L	0.001	0.002	0.055	0.044	ND	ND	ND	0.031	0.019	0.017	ND	ND	ND	83.6-108
DDD(o,p')	ug/L	0.001	0.002	0.009	ND	ND	ND	ND	ND	0.009	ND	ND	ND	ND	78.7-103
DDD(p,p')	ug/L	0.001	0.002	0.017	0.002	ND	ND	ND	ND	0.020	ND	ND	ND	ND	79-115
DDE(o,p')	ug/L	0.001	0.002	ND	ND	ND	ND	ND	ND	0.011	ND	ND	ND	ND	70-103
DDE(p,p')	ug/L	0.001	0.002	0.107	0.007	ND	0.01	0.077	0.023	0.187	0.007	ND	0.064	ND	79.3-112
DDT(o,p')	ug/L	0.001	0.002	ND	ND	ND	ND	ND	ND	0.027	ND	ND	0.016	ND	72.9-117
DDT(p,p')	ug/L	0.002	0.005	0.039	ND	0.056	ND	0.048	0.015	0.094	ND	0.027	0.021	ND	84-129
Dieldrin	ug/L	0.001	0.002	ND	ND	ND	ND	0.019	0.019	0.036	0.011	ND	ND	ND	90.5-109
Endrin	ug/L	0.001	0.002	ND	ND	ND	ND	ND	ND	ND	ND	0.022	0.027	ND	80.2-124
Nonachlor, trans-	ug/L	0.001	0.002	ND	ND	ND	ND	ND	ND	ND	ND	0.017	0.017	ND	78.3-102
Dibromooctafluorobiphenyl	Percent Surrogate Recovery			71.4	91.8	88	95.6	95.4	106	79.4	91.4	109	99.5	109	NA
Carbamates															
Carbaryl	ug/L	0.010	0.020	ND	ND	ND	ND	0.059	0.053	ND	ND	ND	ND	ND	69.5-99.7
Carbofuran	ug/L	0.010	0.020	ND	ND	ND	ND	0.350	0.021	ND	ND	ND	ND	ND	78.6-119
Methomyl	ug/L	0.010	0.020	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.440	82.9-105

The sediment sample collected at the SV-03 inlet October 30 caused complete amphipod mortality (Table 15), and a TIE was initiated. The addition of Ambersorb to the sediment did not reduce toxicity, but the addition of powdered coconut charcoal increased the survival to 50%, indicating toxicity was caused by an organic (Table 18). In separate treatments, the addition of carboxylesterase to the overlying water increased survival to 78%, and the addition of BSA did not reduce toxicity, indicating toxicity caused by a pyrethroid. The Ambersorb eluate was toxic (0% survival), further supporting the implication of an organic compound.

Table 18. Results of toxicity identification evaluation of site SV-03 sediment sample.

	Solid-Phase TIE		Amendment Elution	
Treatment	Mean	SD	Mean	SD
SV-03 A	0	0		
SV-03 A (10% Ambersorb)	0	0	0	0
Control (10% Ambersorb)	96	5	43	6
SV-03 A (10% PCC)	50	19		
Control (10% PCC)	100	0		
SV-03 A (10% SIR-300)	0	0		
Control (10% SIR-300)	98	4		
SV-03 A (Enzyme)	78	15		
Control (Enzyme)	92	8		
SV-03 A (BSA)	0	0		
Control (BSA)	98	4		
SV-03 A (10% Control)	0	0		
Sediment Control	96	9		
Ambersorb Elution Control			100	0

The baseline interstitial water extracted from SV-03 sediment contained 6.3 TUs, but only 234 ng/L diazinon (Table 19). This concentration of diazinon was significantly less than the LC50 of 6210 ng/L for the amphipod *H. azteca* (Phipps et al. 1995). The HLB column reduced the toxicity to 2.4 TUs, and the HLB eluate returned the toxicity to clean dilution water. Addition of the enzyme to the interstitial water completely removed toxicity, while the addition of BSA reduced toxicity by 1.5 TUs. Addition of PBO increased toxicity to 20 TUs, and the addition of carboxylesterase and PBO in combination reduced the toxicity to 3.5 TUs. These results provide strong evidence that toxicity was caused by a pyrethroid. Subsequent GCMS chemical analysis revealed a very high concentration (66.4 ng/g) of the pyrethroid pesticide cypermethrin (Table 14b). The pyrethroid pesticide lambda-cyhalothrin was also present at toxic

concentrations in this sample: 18.8 ng/g dry weight, compared to an LC50 of 6 ng/g (Amweg et al. 2005), and an organic carbon normalized concentration of 0.79 ng/g OC, compared to an LC50 value of 0.45 ng/g OC.

Table 19. Results of toxicity identification evaluation of site SV-03 porewater sample.

SV-03 A	Percent Sample											
	0%		10%		25%		50%		100%		Toxic	Diazinon
Treatment	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Units	ng/L
Baseline	93	12	93	12	0	0	0	0	0	0	6.3	234
HLB Column	100	0	100	0	100	0	27	31	0	0	2.4	<RL
HLB Eluate	93	12	67	31	20	20	0	0	0	0	6.8	177
Carboxylesterase	93	12	87	23	94	10	100	0	87	12	<1	133
BSA	100	0	100	0	33	42	0	0	0	0	4.8	163
PBO	100	0	0	0	0	0	0	0	0	0	20.0	198
Carboxylesterase/PBO	100	0	100	0	47	23	30	26	0	0	3.5	189

Pesticide reduction at site SV-03

The vegetated treatment system at site SV-03 had a different pesticide profile than that at G-09. Chlorpyrifos was seldom detected, and diazinon and dimethoate were the most commonly detected OP pesticides. Diazinon was found at concentrations near the median lethal concentration for *C. dubia* in four of the five surveys, and in the October 4 survey, nearly 9 ug/L diazinon was measured at the inlet, about 25 times the LC50 for diazinon toxicity to *C. dubia* (Figure 29). In this instance, the concentration at the inlet was much higher than at the outlet, but whether this is due to treatment in the VTS, dilution of a pulse in the VTS, or capture of a pulse at the inlet is uncertain. A similar pattern was seen for the OP pesticide dimethoate (Figure 30).

Figure 30. Diazinon concentrations at the inlet (A) and outlet (B) of the VTS at site SV-03.

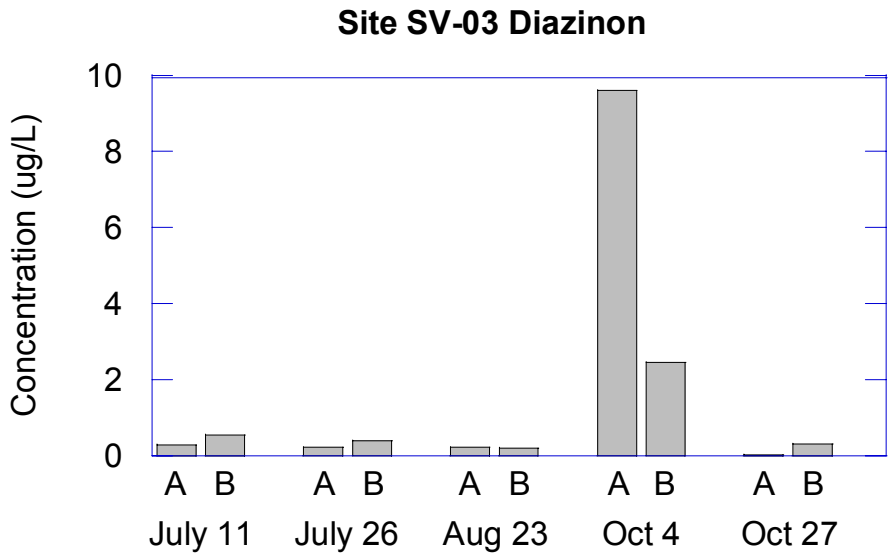
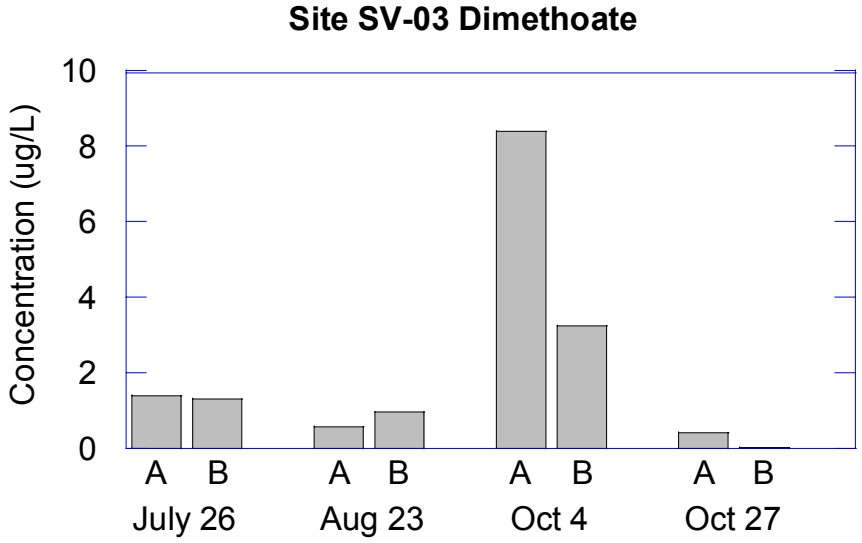
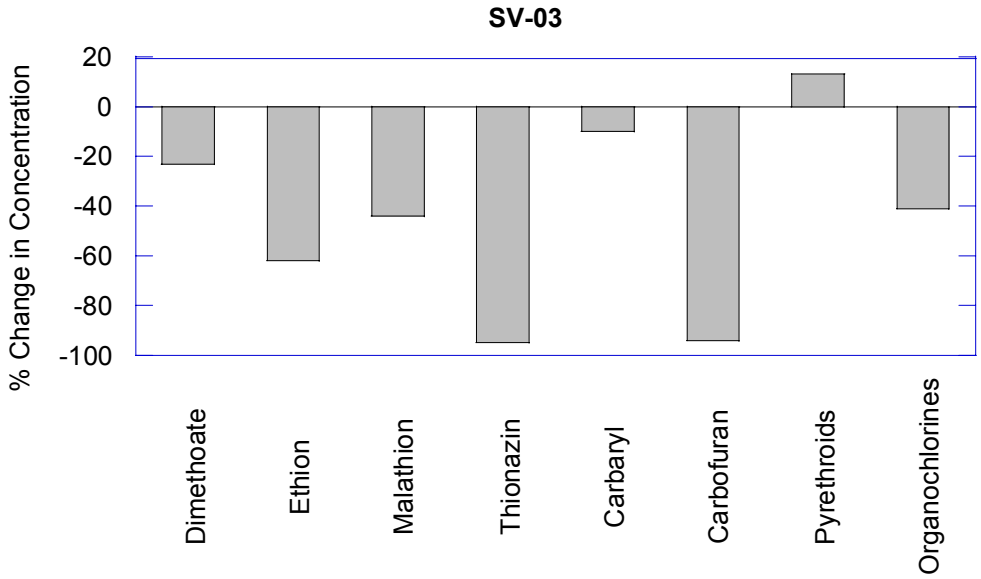


Figure 31. Dimethoate concentrations at the inlet (A) and outlet (B) of the VTS at site SV-03.



As with the data from site G-09, the SV-03 data were averaged to look at all chemicals that were detected at the inflow during all surveys, and examine the mean difference in their concentrations at stations A and C (Figure 31). The diazinon trend can be seen in the previous figure (Figure 29). For the other OP pesticides and the carbamates (carbaryl and carbofuran), the average concentrations were lower in the outlet samples than in the corresponding inlet samples. The same was true for the organochlorines, which were generally found at low concentrations. The pyrethroids were found at higher concentrations in water samples from the outlet, but this is mainly due to variability around very low water concentrations near the detection limits. Pyrethroids have very low water solubilities, and are generally found at much higher concentrations in sediments. In the SV-03 VTS, as discussed above, the pyrethroid pesticide cypermethrin was found at high concentrations in the sediment, and there was strong TIE evidence implicating it as the cause of sediment toxicity. It is possible that elevated pyrethroids at the VTS outlet may be associated with flux from the sediments; but, again, it is difficult to draw conclusions based on very low water column concentrations.

Figure 32. Change in concentration for all chemicals detected in water at both the inflow and outflow of the VTS at SV-03. Values are means for all surveys in which chemicals were detected. Chlorpyrifos, diazinon, permethrin, dacthal, DDE(p,p') and methomyl were detected at the outflow but not in the inflow from the same survey.



Paired-sample t-tests were used to test the null hypothesis that pond inlet and outlet concentrations were the same. Statistically significant differences were found only for organochlorines ($p = 0.001$). The null hypothesis could not be rejected for the following categories: all chemicals combined, total OPs, diazinon, carbamates or pyrethroids.

Sediment chemistry

Trends in sediment chemical concentrations at SV-03 were most pronounced for the pyrethroid pesticides. Cypermethrin and lambda-cyhalothrin, which were both implicated as causes of sediment toxicity, were measured at higher concentrations in sediments collected at the SV-03 VTS inlet than at the outlet. Cypermethrin declined from 66.4 ng/g to below the detection limit of 2 ng/g, and lambda-cyhalothrin declined from 18.8 ng/g to 5.75 ng/g (just below the published LC50 of 6 ng/g; Amweg et al. 2005). There was little change in the already lower concentrations of other pyrethroids, and there was only a slight decrease in the total organic carbon concentration (2.37% inlet, 2.07% outlet). Organophosphate compounds were not detected in SV-03 sediments, and organochlorine pesticide concentrations were generally low, with mixed trends between inlet and outlet. Total DDT declined from an inlet sediment concentration of 546 ng/g to an outlet concentration of 470 ng/g. As with the G-09 sediments, the DDT concentrations likely reflect typical variability for this ubiquitous compound.

On-farm vegetated treatment system hydrology and reductions in turbidity, nitrate, phosphate, and diazinon

The hydrology of the farms sites was assessed to facilitate interpretation of pesticide concentration and toxicity data. No rain events or stormwater runoff occurred during the study. Discharge to the ponds was restricted to irrigation runoff (tailwater). No evidence of groundwater seepage into the ponds was observed, and tile drainage was not used on either farm.

The hydrology of tailwater on the two sites varied considerably. G-09 was characterized by continuous and relatively consistent flow rates, which averaged 0.24 cubic feet per second. Discharge was estimated by measuring the depth of water at the culvert that serves as the pond inlet, which exhibited weir flow conditions. Flow was determined using the broad crested weir

equation and a rating curve generated using HEC-RAS 3.1.3 software (Brunner, 2002). The estimated error associated with this approach is less than 25 percent. Inlet control was consistently observed. Backwater effects or submerged inlet conditions were not observed. The bathymetry and volume of the pond was estimated based on soundings measurements and verbal communication with the cooperator.

At G-09, limited data (n=5) collected as part of this work suggests that water quality parameters were improved by this practice. For each parameter the average change between the inflow and outflow was determined. Mean turbidity was reduced by 92 percent, and mean nitrate concentrations by 20 percent. Mean orthophosphate concentrations increased by one percent. Statistical inference is limited by the small sample size.

SV-03 was characterized by highly variable inlet flow rates. Irrigation events typically lasted about 12 hours, and runoff stopped within 4 hours of the end of irrigation. Irrigation events occurred as frequently as three times in four days, and as infrequently as once in five days. Absent an irrigation event, no flow occurred. Flow was measured at an HS flume (Brackensiek et al., 1979) using stage measured by hand during the first three months of the study and with a continuous water level logger during October 2006. The estimated error associated with this approach is less than 10 percent. Outflow was estimated by measuring the depth of water at the culvert that serves as the outlet of the pond and calculating flow using the broad crested weir equation and a rating curve generated using HEC-RAS 3.1.3 software (Brunner, 2002). The estimated error associated with this approach is less than 25 percent. Backwater effects or submerged inlet conditions were not encountered. The mean flow rate measured during the study period (including periods of no flow) was 0.035 CFS. The bathymetry and volume of the ponds was estimated based on soundings measurements and verbal communication with the cooperator. These data are summarized in Table 13.

At SV-03, limited data (n=5) collected as part of this work suggests that water quality parameters were improved by this practice. For each parameter the average change between the inflow and outflow was determined. Turbidity at the inflow consistently exceeded the instrument range of 1000 NTU. For the purpose of calculating average change, 1000 NTU was used for those data. Mean turbidity was reduced by 96 percent, mean nitrate concentrations were reduced by 38 percent, and mean orthophosphate concentrations were reduced by 94 percent. Statistical inference is limited by the small sample size.

Table 20. Summary of hydrologic and water quality data for sites G-09 and SV-03.

Hydrologic data summary		
	G-09	**SV-03**
production type	greenhouses	row crops
drainage area (acres)	85	120
vegetated treatment system area including perimeter roads (acres)	0.50	0.33
vegetated treatment system volume (acre-feet)	1.1	0.52
mean flow rate (cubic feet per second)	0.24	0.035
mean flow rate (gallons per minute)	110	18
nominal residence time (flow rate/volume in days)	7.5	2.3
turbidity (mean change inflow to outflow, n=5)	-92 %	-96 %
nitrate (mean change inflow to outflow, n=5)	-20 %	-38 %
phosphate (mean change inflow to outflow, n=5)	+1 %	-94 %

Pesticide degradation is a function of time, and degradation processes operate on time scales of days to years. In order to interpret observed changes in concentration, it is important to understand the duration of pesticide exposure to particular environmental conditions.

The time a parcel of water spends in a treatment system is its residence time. The nominal or theoretical residence time is a theoretical value based on the assumption that short circuiting and mixing do not occur. It is determined as the quotient of system volume divided by flow rate.

$$t_d = \frac{V}{Q} \qquad (1)$$

where t_d is the nominal residence time (T)

V is the volume (L^3), and

Q is the discharge rate (L^3/T).

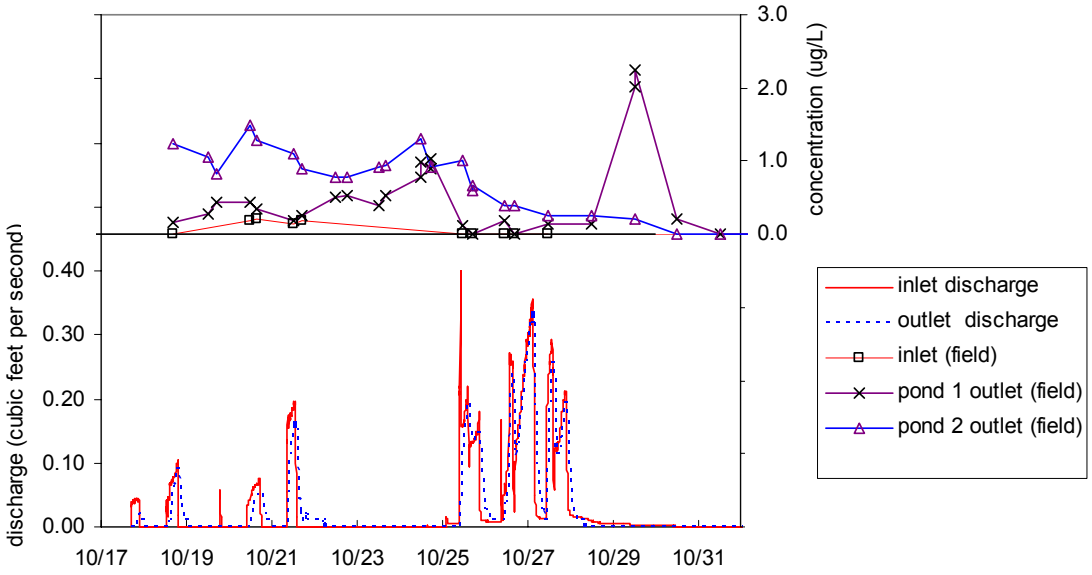
The actual residence time is a distribution function that results from the combined effects of preferential flow paths and mixing processes. Shear along the bed, banks and plant surfaces, turbulence, and diffusion cause different parts of the water body to contribute unequally to the transport of a constituent. If a spike of a conservative tracer is injected at the inlet, a plot of concentration at the outlet typically reveals that the bulk of the constituent arrives before the

theoretical residence time, the peak concentration is substantially lower than the spike concentration, and low concentrations persist for an extended period as the residual constituent is gradually flushed through the system. This is illustrated graphically below.

In this study reductions in diazinon concentrations were observed at SV-03, leading to the question: are concentration reductions the result of treatment or dilution? One approach is to measure flow and concentration at the inflow and outflow and construct a mass balance of the constituent with the VTS as a control volume. This was performed during October 2006.

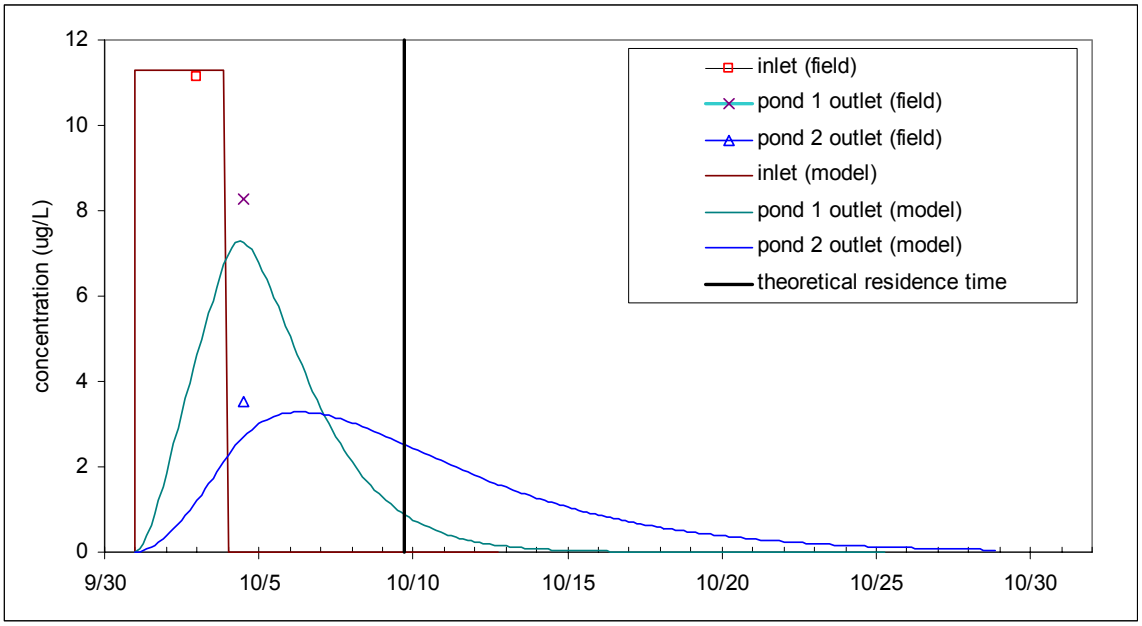
The continuous flow record, presented in Figure 32, demonstrates a rapid response between inflow and outflow: the water levels in the ponds rise and fall rapidly, but the ponds retain one to two meters of water between tailwater events. Simplified models of wet detention basins (discussed in Haan et al 1994) assume that incoming water displaces resident water. This is not evident in the record of diazinon concentrations. During this period, inflowing diazinon concentrations were consistently low. Concentrations at the outlet of pond 1 were also low while flow was occurring, but were higher during periods of no flow. This indicates during quiescent conditions diazinon was entering the water column at the outlet of pond 1. Samples of the bed sediment were non-detect for diazinon, and diazinon was not applied to the ponds, suggesting that the likely source was mixing from backwater areas or the interstitial water among the plants. Desorption from plant surfaces is also a possibility.

Figure 33. Time series of flow and diazinon concentration at SV-03. Diazinon concentrations were measured at the inlet, outlet of pond 1 and outlet to pond 2. Few inputs of diazinon occurred during this period. Concentrations at the outlet of pond 1 were lowest during flow events, suggesting that low concentration water was passing through the pond, and that stagnant water with higher concentrations mixed through the water column under quiescent conditions. Concentrations at the outlet of pond 2 declined continuously during the period, suggesting more complete mixing and the gradual decline in concentrations. The data are inconclusive whether degradation is occurring or if concentration changes are the result of dilution.



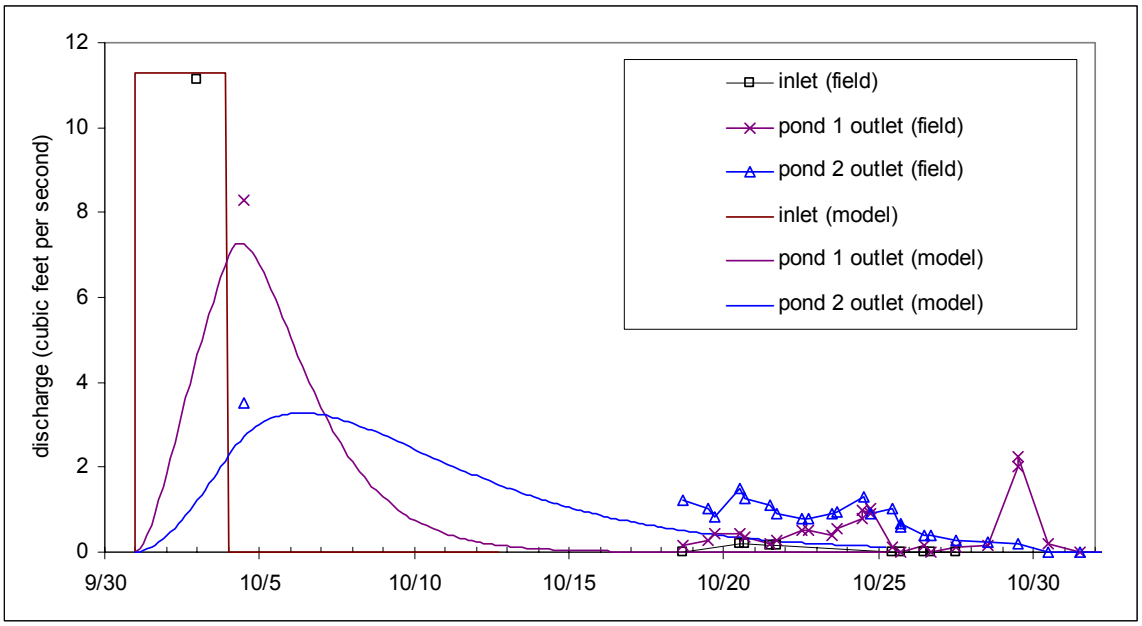
In order to understand mixing in this type of system, the standard approach to determine the residence time distribution by injecting a conservative tracer such as bromide at the inlet and observing concentrations at the outlet. That work was beyond the scope of this investigation. A tank-in-series model was developed (after Haan et al 1994), which represents the water body as a series of completely mixed compartments (continuously stirred tank reactors). The application of this model contains numerous assumptions, including steady state (constant) flow, which was set to the mean flow rate. It is intended as a general illustration of mixing and dilution processes rather than an accurate representation of this system. The model was calibrated to data measured on 10/4/06 and 10/5/06 assuming no reaction or degradation of diazinon. The number of reactors was set to two (n=2), and the percent of dead space was set to zero. Model results are shown below (figure 33).

Figure 34. Model of a non-reactive tracer at SV-3 compared to observed inflow and outflow concentrations of diazinon. Dilution and mixing alone is sufficient to explain the observed changes in concentration. The model contains numerous simplifying assumptions, and is intended as a general illustration rather than as an accurate representation of this system. The theoretical residence time indicated is 7.2 days from the midpoint of the modeled spike at the inlet.



The result of these simulations were superimposed on the continuous dataset collected at the end of October, and illustrate the dominant effect of the concentration spike observed on 10/5/06 on data collected through the rest of the month (figure 34).

Figure 35. Results from hydraulic mixing model of a non-reactive tracer and field data. The spike of diazinon on October 4 and 5 is sufficient to explain a substantial portion of elevated concentrations observed later in the month, if diazinon behaves as a non-reactive tracer. Other sources and sinks of diazinon may have been operating during this period. The model was calibrated to concentration measurements made on 10/4 and 10/5. These results demonstrate the importance of mixing and dilution to transport processes. The model contains numerous simplifying assumptions, and is intended as a general illustration of processes rather than an accurate representation of this system.



One indication of this modeling effort is that degradation of diazinon is not required to explain the apparent decrease in concentrations from inflow to outflow. It is possible and perhaps likely that other loading events and degradation of diazinon are occurring during this period.

The modeling demonstrates that at site SV-03 generating a composite sample at the inflow on three successive days and sampling the outflow on the third day was a reasonable approach to capture some of the temporal variability in the system.

Over a growing season, various pesticides are applied to a typical production block. Some of these may be mobilized by runoff and leave the field in concentrations sufficiently elevated to impact receiving waters. The mixing model illustrates the service wet retention basins provide as a location where in addition to treatment, dilution occurs, reducing peak concentrations downstream.

Neither the model nor the field data characterize the system satisfactorily. Assessment of these types of systems will be improved by the use of tracer studies to characterize the role of mixing in constituent transport, and by frequent measurements of discharge and constituent concentrations. The temporal variability of loading events also speaks to the value of *in situ* toxicity testing.

Pesticide concentrations related to suspended sediment and turbidity

In addition to hydrolysis, photolysis, and binding/breakdown on plant surfaces, one of the primary processes for removing pesticides from water in vegetated treatment systems is binding of hydrophobic compounds with suspended sediments, and subsequent settling of these particles in the slow moving waters of the ponds or wetlands. We investigated the relationship between water concentrations of various pesticides and either suspended sediment concentration (Tembladero Slough) or turbidity (vegetated ponds). All water sample chemical analyses were made on extracts of unfiltered samples.

In Tembladero Slough treatment system water samples, there was no obvious relationship between concentrations of diazinon and suspended sediments (Figure 35). This might be expected given the relatively high solubility of diazinon. While there were only four samples to analyze, the trend with organochlorines was as expected: higher concentrations were associated with higher suspended sediments (Figure 36). The same relationship was expected for pyrethroids, but the opposite was seen (Figure 37). These results should be viewed with caution because of the very low pyrethroid concentrations, most were only about twice the detection limit. It is also worth noting that pesticide reductions are also affected by sorption to plants (OCs and pyrethroids), or by chemical breakdown (diazinon). These processes may confound interpretation of the suspended particle/pesticide relationships.

Figure 36. Relationship between suspended sediment concentration and diazinon concentrations in the constructed channel and wetland at Tembladero Slough.

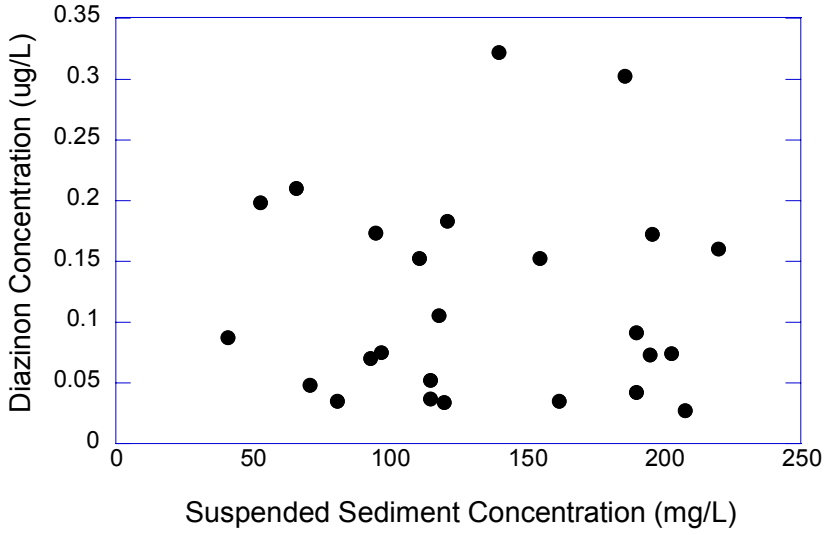


Figure 37. Relationship between suspended sediment concentration and sum organochlorine concentrations in the constructed channel and wetland at Tembladero Slough.

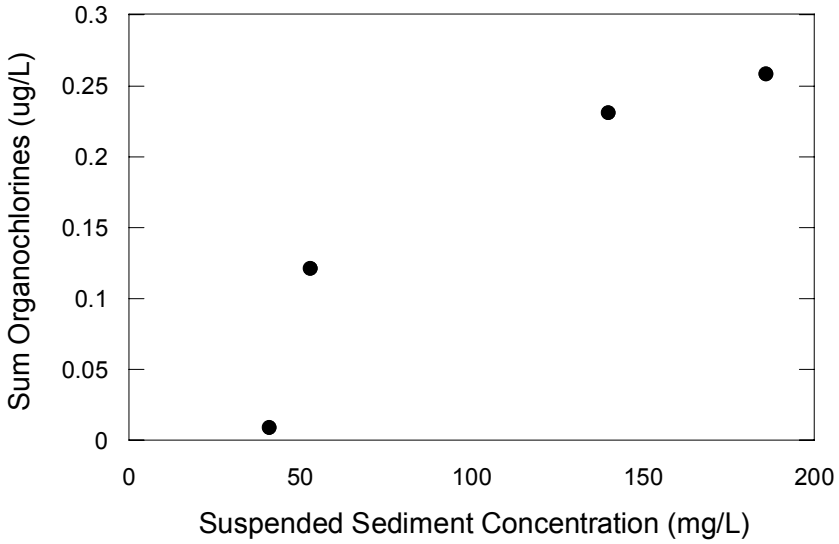
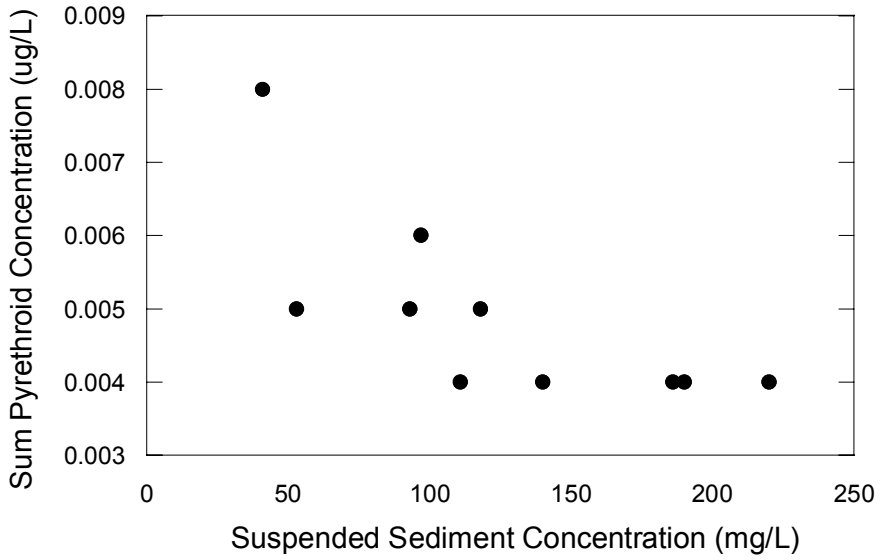


Figure 38. Relationship between suspended sediment concentration and pyrethroid pesticide concentrations in the constructed channel and wetland at Tembladero Slough.



With water samples from site G-09, there is again no obvious relationship between concentrations of diazinon and turbidity (Figure 38). Chlorpyrifos, which was found at relatively high concentrations, appears to be higher in samples with higher turbidity. Total pyrethroid concentrations in G-09 samples also increased with increasing turbidity (Figure 37). Note that four of these samples had relatively high water pyrethroid concentrations (> 20ng/L). All three of these figures highlight the same single sample that had high turbidity and high concentrations of all three pesticide types.

Figure 39. Relationship between turbidity and diazinon concentrations in the treatment pond at site G-09.

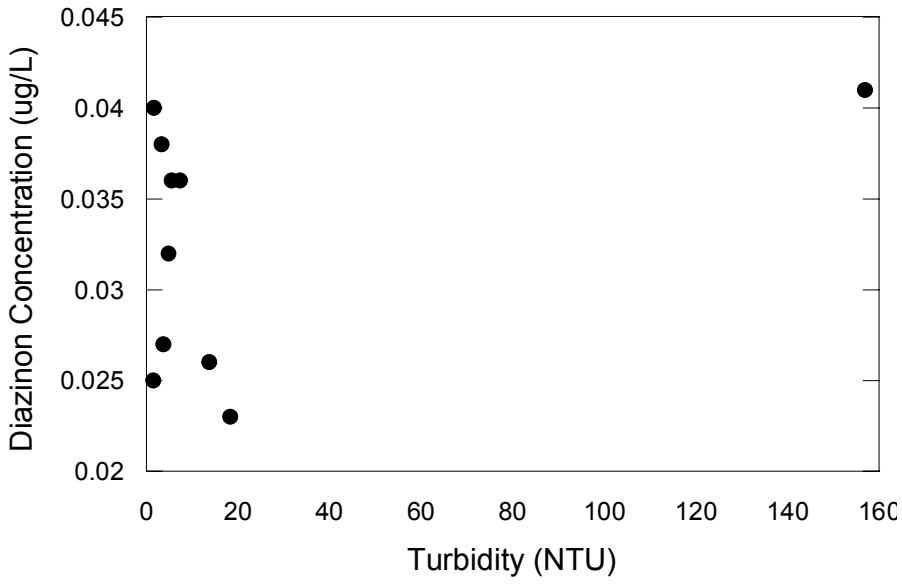


Figure 40. Relationship between turbidity and chlorpyrifos concentrations in the treatment pond at site G-09.

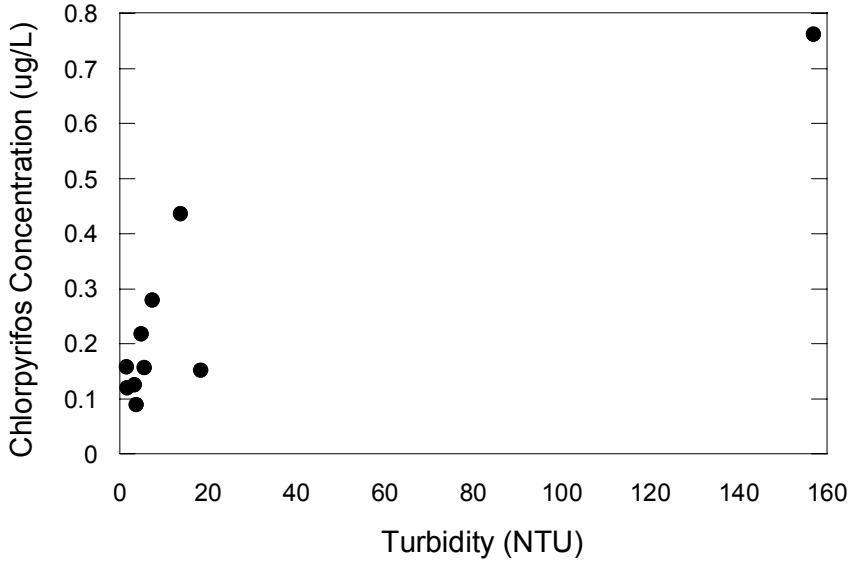
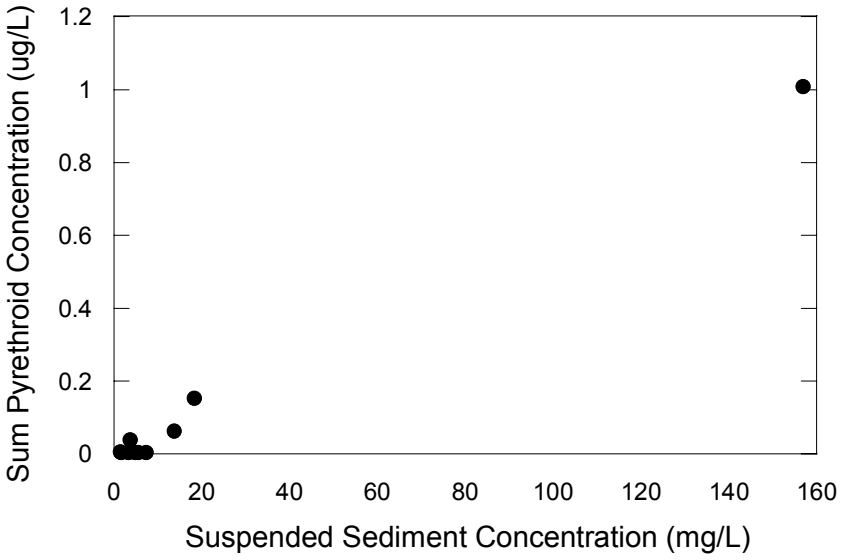


Figure 41. Relationship between turbidity and pyrethroid pesticide concentrations in the treatment pond at site G-09.



A number of water samples from the ponds at site SV-03 had turbidity readings above the maximum measurable by the field meter (1000 NTU). Given that, diazinon shows no clear trend, but there was high turbidity in the sample that had extremely high diazinon (Figure 41). The hydrophobic organochlorines are relatively high in the high turbidity samples, but were present in other samples as well (Figure 42). Again, the role of plants as sorption sites should not be ignored when considering this analysis. This study was not designed to elucidate the mechanisms of pesticide reduction in the VTSs, but these results provide some preliminary relationships where possible.

Figure 42. Relationship between turbidity and diazinon concentrations in the treatment pond at site SV-03. Note that 1000 NTU is the maximum for the instrument.

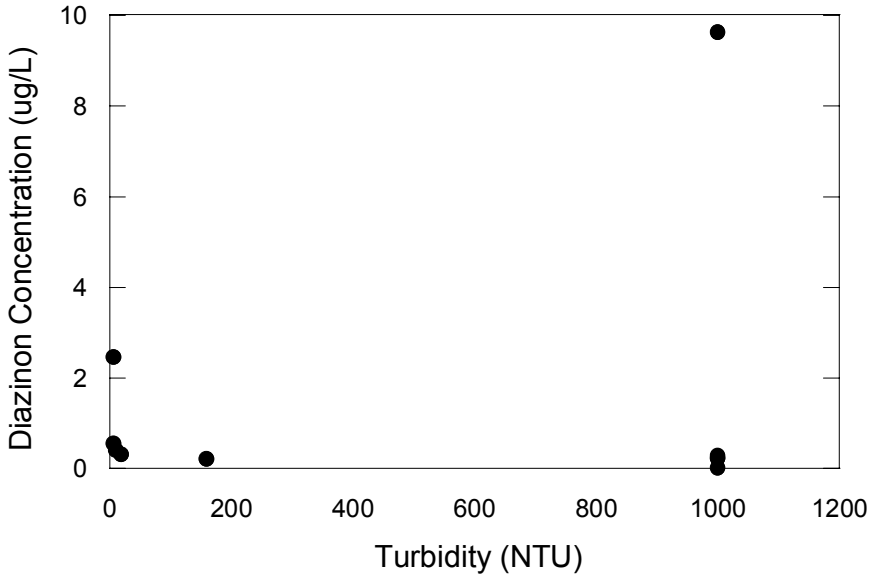
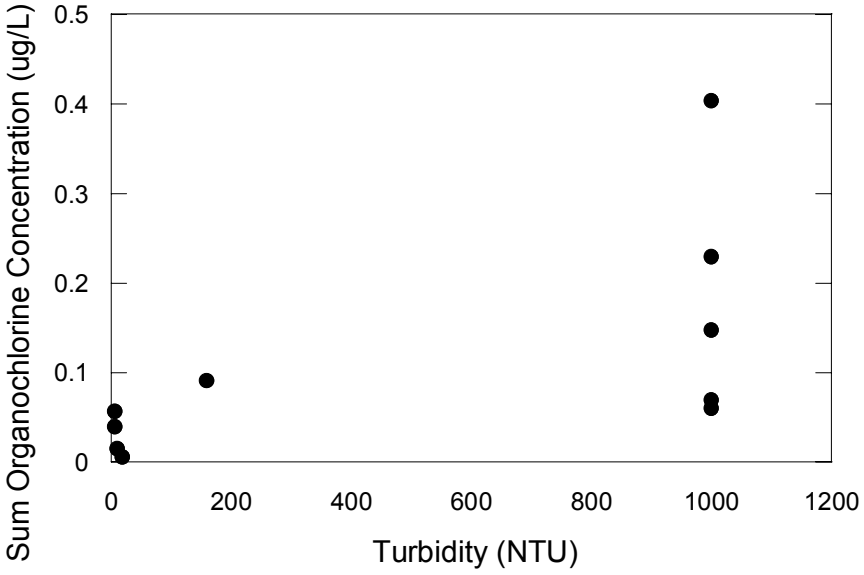


Figure 43. Relationship between turbidity and organochlorine pesticide concentrations in the treatment pond at site SV-03.



Conclusions

This study evaluated the effectiveness of nutrient and pesticide reduction in a constructed wetland vegetated treatment system designed to treat runoff at a watershed scale, and in two on-farm VTS ponds, that were built by cooperators to fit within the operating constraints of commercial agricultural production. These systems were not originally optimized for this evaluation. The constructed wetland received inflows that had higher dissolved salt content than was tolerated by the sensitive toxicity test organism chosen for the study. The on-farm VTS systems had multiple inputs and pulsed flows that greatly complicated analysis of system hydrology. Despite these constraints, a number of useful conclusions can be drawn from the data gathered.

Tembladero Slough VTS

1. Water samples from the Tembladero Slough (which was pumped into the constructed wetland VTS) were toxic to *C. dubia*.
2. This toxicity appeared related to total organophosphate pesticide concentrations.
3. In surveys using *C. dubia*, toxicity and pesticide concentrations decreased with distance traveled through the constructed wetland.
4. Pesticide reductions tended to increase with longer residence time in the system.
5. Pesticide concentrations for organochlorine pesticides were related to suspended particle concentrations.
6. Pesticide reduction with distance from the inlet was not observed in all surveys; but when a diazinon-laden parcel of water was tracked through the system over its three-day residence time, diazinon concentrations decreased, as did toxicity.

On-Farm VTS Ponds

1. Inflows to VTS pond G-09 were highly toxic to *C. dubia* in all samples.
2. TIEs and subsequent chemical analyses indicated that chlorpyrifos was responsible for toxicity in water and sediment samples from VTS site G-09, and that the pyrethroid pesticide permethrin was also responsible for sediment toxicity.

3. High influent concentrations of chlorpyrifos at G-09 decreased to much lower concentrations at the VTS outlet in all five surveys.
4. Concentrations of most pesticide classes were lower at the G-09 outlet than at the inlet, indicating overall VTS effectiveness in reducing pesticide concentrations.
5. At the SV-03 VTS site, influent samples were toxic to *C. dubia* in four of five surveys.
6. TIEs indicated that toxicity was due to diazinon, which occurred at concentrations as high as 9.6 ug/L.
7. There was strong TIE evidence that sediment sample toxicity at this site was due to high concentrations of the pyrethroid pesticides lambda-cyhalothrin and cypermethrin.
8. Diazinon concentrations generally did not decrease in the VTS, though the high (9.6 ug/L) diazinon inflow pulse was not seen at the system outlet.
9. Concentrations of most pesticide classes were lower at the SV-03 outlet than at the inlet, again indicating overall VTS effectiveness in reducing pesticide concentrations.
10. A focused study of VTS hydrology and diazinon concentration at SV-03 indicated that complex in-pond dilution processes were responsible for dampening pulse inflow peak concentrations, so that outflows had generally lower concentrations.

The overall conclusion that can be drawn from these analyses is that these VTS systems were effective at markedly reducing pesticide and nitrate concentrations. Most pesticides showed declines in most cases. Water soluble pesticides such as diazinon may not be broken down within the residence times observed in these VTS systems; but focused studies showed notable reductions when diazinon-laden parcels were carefully tracked, and when hydrology was thoroughly investigated.

Further work should include thorough initial hydrologic characterizations to allow more precise measurements, and mass balance investigations to determine which components of the systems are responsible for pesticide and nutrient reductions. These studies will greatly increase our knowledge of system properties, and allow the design of increasingly efficient conservation practices to mitigate the effects of non-point source runoff to the critical aquatic habitats of the central coast.

REFERENCES

Amweg EL, Weston DP, Ureda NM. 2005. Use and toxicity ofpyrethroid pesticides in the Central Valley, CA, USA. *Environ Toxicol Chem* 24:966–972.

Anderson BA, Phillips BM, Hunt JW, Connor V, Richard N, Tjeerdema RS. 2006. Identifying primary stressors impacting macroinvertebrates in the Salinas River (California, USA): relative effects of pesticides and suspended particles. Environ Poll 141: 402-408.

Anderson BS, et al. 2006. Evidence of pesticide impacts in the Santa Maria River watershed (California, USA). *Environ Toxicol Chem* 25(4) 1160 – 1170.

Anderson BS, et al. 2003. Integrated assessments of the impacts of agricultural drain water in the Salinas River (California, USA). *Environ Poll* 124: 523-532.

Bailey, H. C., Miller, J. L., Miller, M. J., Wiborg, L. C., Deanovic, L. and Shed, T. 1997. Joint acute toxicity of diazinon and chlorpyrifos to *Ceriodaphnia dubia*, *Environ. Toxicol. Chem.* **16**: 2304–2308.

Bailey HC, DiGiorgio C, Kroll K, Miller JL, Hinton DE, Starrett G. 1996. Development of procedures for identifying pesticide toxicity in ambient waters: carbofuran, diazinon, chlorpyrifos. Environ Toxicol Chem 15: 837-845.

Beusen, J.M., and B. Neven. 1989. Toxicity of dimethoate to *Daphnia magna* and freshwater fish. Bull. Environ. Contam. Toxicol. 42(1):126-133.

Brakensiek, DL, HB Osborn, and WJ Rawls. 1979. (Editors and Coordinators). Field manual for research in agricultural hydrology. USDA Agricultural Handbook 224, Revised, February 1979. 547 p.

Brown RP, Landre AM, Miller JA, Kirk HD, Hugo JM. 1997. Toxicity of sediment-associated chlorpyrifos with the freshwater invertebrates *Hyalella azteca* (amphipod) and *Chironomus tentans* (midge). DECO-ES-3036. Health and Environmental Research Laboratories, Dow Chemical, Midland, MI, USA.

Brunner, GW 2002. HEC-RAS, River analysis system hydraulic reference manual: U.S. Army Corps of Engineers Hydrologic Engineering Center, CPD-69, November 2002, Version 3.1, 350 p.

Carey, J, Cook, P, Giesy, J, Hodson, P, Muir, D. Owens, W, Solomon, K. 1998. Ecological risk assessment of the chlorinated organic chemicals. SETAC press, Pensacola, FL.

Coats, JR, Symonik, DM, Bradbury, SP, Dyer, SD, Timson, LK, Atchison, GJ. 1989. Toxicology of synthetic pyrethroids in aquatic organisms: an overview. Environ Toxicol Chem 8: 671-680.

Giesy JP, Solomon KR, Coats JR, Dixon KR, Giddings JM, Kenaga EE. Chlorpyrifos: Ecological risk assessment in North American aquatic environments. *Rev Environ Contam Toxicol* 160:1–129.

Giddings JM, Solomon KR, Maund SJ. 2001. Probabilistic risk assessment of cotton pyrethroids: II. Aquatic mesocosm and field studies. *Environ Toxicol Chem* 20:660–668.

Han, CT, BJ Barfield, and JC Hayes. 1994. Design Hydrology and Sedimentology for Small Catchments. Academic Press, San Diego, CA. 588 p.

Hendley, P, Dobbs, M, Warington, J. 2006. Synthetic pyrethroids in the environment: an overview of environmental behavior and ongoing aquatic ecological risk assessments. Proceedings of the 27th annual meeting of the Society of Environmental Toxicology and Chemistry (SETAC), Montreal, Canada, Nov. 5 – 9, 2006.

Hunt JW, Anderson BS, Phillips BM, Nicely PA, Tjeerdema RS, Puckett HM, Stephenson M, Worcester K, de Vlaming V. 2003. Ambient toxicity due to chlorpyrifos and diazinon in a central California coastal watershed. Environ Monitor Assess 82: 83-112.

Hunt JW, et al.. 1999. Patterns of aquatic toxicity in an agriculturally dominated coastal watershed of California. *Agricul. Ecosyst. Environ*. 75: 75-91.

Marshack, J. 2007. A compilation of water quality goals. SWRCB Website: http://www.swrcb.ca.gov/rwqcb5/available_documents/wq_goals/index.html#anchor274991

Moore, MT, Bennett, ER, Cooper, CM, Smith, S, Shields, FD, Milam, CD, Farris, JL. 2001. Transport and fate of atrazine and lambda-cyhalothrin in a vegetated drainage ditch in the Mississippi Delta. Agr. Ecosyst. Env. 87: 309-314.

Novartis Crop Protection, Inc. 1997. An ecological risk assessment of diazinon in the Sacramento and San Joaquin River basins. Technical report: 11/97, Novartis Inc., Greensboro, NC.

Pantani, C., Pannunzio, G., DeChristofaro, M., Novelli, A.A., and Salvatori, M.: 1997, 'Comparative Acute Toxicity of Some Pesticides, Metals, and Surfactants to *Gammarus italicus* and *Echinogammarus tibaldi*.' *Bull. Environ. Contam. Toxicol*. 59, 963-967.

Phipps GL, Mattson VR, Ankley GT. 1995. The relative sensitivity of three benthic test species to 10 chemicals. Arch Environ Toxicol Chem 28: 281-286.

Schulz, R. 2004. Field studies and exposure, effects, and risk mitigation of aquatic non-point source insecticide pollution: a review. J. Environ. Qual. 33: 419 – 449.

Sherrard, RM, Bearr, JS, Murray-Gulde, CL, Rodgers, JH, Shah, YT. 2004. Feasibility of constructed wetlands for removing chlorothalonil and chlorpyrifos from aqueous mixtures. Environ Poll. 127: 385-394.

Sullivan JJ, Goh KS. 2000. Evaluation and validation of a commercial ELISA for diazinon in surface waters. J Agric Food Chem 48: 4071-4078.

U.S. Environmental Protection Agency. 1993. Methods for aquatic toxicity identification evaluations. Phase II Toxicity Identification Procedures for Samples Exhibiting Acute and Chronic Toxicity. EPA 600/R-92/080. Office of Research and Development. Washington, DC.

U.S. Environmental Protection Agency. 1993. Methods for the Determination of Non-conventional Pesticides in Municipal and Industrial Wastewater, Volume I. Revision 1. EPA 821/R-93-010-A. Office Water, Washington, DC.

U.S. Environmental Protection Agency. 2000. Pesticide Ecotoxicity Database (Formerly: Environmental Effects Database (EEDB)); Office of Pesticide Programs; Environmental Fate and Effects Division, U.S.EPA, Washington, D.C.

U.S. Environmental Protection Agency. 2000. Ambient Water Quality Criteria Recommendations—Information Supporting the Development of State and Tribal Nutrient Criteria for Rivers and Streams in Nutrient Ecoregion III. EPA 822-B-00-016. U.S. Environmental Protection Agency, Office of Water, Office of Science and Technology, Health and Ecological Criteria Division, Washington, D.C. December 2000.

U.S. Environmental Protection Agency. 2002. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. EPA-821-R-02-012. Office of Water. Washington, DC.

U.S. Environmental Protection Agency. 2007. Aquatic life benchmarks for 71 pesticides or degradation products. Office of Pesticide Programs: http://www.epa.gov/oppefed1/ecorisk_ders/aquatic_life_benchmark.htm.

Watanabe, H, Grismer, ME. 2001. Diazinon transport through inter-row vegetative filter strips: micro-ecosystem modeling. J. Hydrology 247: 183-199.

Wheelock, C.E., Miller, J.L., Miller, M.J., Gee, S.J., Shan, G., Hammock, B.D. 2004. Development of toxicity identification evaluation procedure for pyrethroid detection using esterase activity. *Environ Toxicol Chem* 23: 2699-2708.

Wheelock C, Miller JL, Miller MJ, Phillips BM, Huntley SA, Gee SJ, Tjeerdema RS, Hammock BD. 2006. Use of carboxylesterase activity to remove pyrethroid-associated toxicity to *Ceriodaphnia dubia* and *Hyalella azteca* in toxicity identification evaluations (TIEs). Environ Toxicol Chem 25: 973-984.

Xu, S. 2000. Environmental fate of carbaryl. Technical memorandum, California Department of pesticide regulation. http://www.cdpr.ca.gov/docs/empm/pubs/fatememo/carbaryl.pdf