

# Optimization of an Integrated Vegetated Treatment System Incorporating Landguard A900 Enzyme: Reduction of Water Toxicity Caused by Organophosphate and Pyrethroid Pesticides

## Final Report

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## Executive Summary

The agricultural fields of Monterey County contain a \$4 billion/year industry that produces much of the nation's lettuce, strawberries, crucifer crops and grapes. Runoff from irrigated agriculture contributes a significant amount of water to local stream flow, and several studies have measured significant pesticide concentrations and biological impacts in the Salinas River and its watersheds (Anderson et al., 2003b; Hunt et al., 2003; Phillips et al., 2004). Pesticides in runoff from irrigated vegetable production can also impact invertebrate communities (Anderson et al., 2003a; Liess and von der Ohe, 2005; Anderson et al., 2006b), have adverse impacts on salmonids (Scholz et al., 2000), and lead to food web magnification in fish and wildlife (Pereira et al., 1996). Results of studies conducted throughout California have demonstrated that surface water toxicity is caused by organophosphate and pyrethroid pesticides (Anderson et al., 2011). Developing effective systems for the treatment of runoff is necessary to reduce pesticide loading to aquatic habitats and is an integral requirement of recent Central Coast Water Quality Control Board regulations.

The integrated vegetated treatment system (VTS) in the current study was constructed on a working farm in a narrow linear ditch. This system was previously evaluated by Anderson et al. (2011), and consisted of a short sediment settling basin followed by a vegetated portion, and treatment with Landguard™ OP-A enzyme, which hydrolyzes selected organophosphate pesticides. The results of this study indicated minimal removal of diazinon by sedimentation and vegetation, but complete removal using the Landguard enzyme.

The current project was designed to fine tune the technical specifications for conservation practices that reduce pesticide and nutrient concentrations in tailwater runoff. The first goal of the project was to refine the VTS design by measuring the performance of the vegetated component under different volume scenarios. The second goal of the project was to determine the optimal dose and mixing time of the new Landguard A900 enzyme to completely detoxify representative organophosphate pesticides. This phase of the project was addressed using laboratory spiking experiments. The optimal Landguard dose was then used in the refined VTS. Lastly, Landguard efficacy was tested under conditions with high discharge and short mixing times in a larger drainage receiving agricultural runoff from multiple farms. The latter phase of the project was included to demonstrate the efficiency of pesticide removal in a high loading-high runoff scenario. All field trials were conducted in the VTS and larger drainage ditch during the growing season, using actual irrigation runoff events.

The efficacy of the VTS was tested under different volume scenarios by adjusting a riser that retained water in the vegetated portion of the ditch. Trials were conducted at low and high volume settings. Post treatment water samples collected during the high volume trials were generally less toxic and contained lower concentrations of pesticides than post treatment samples from the low volume trials.

Laboratory experiments were conducted by adding the next generation of Landguard enzyme to water samples spiked with chlorpyrifos and diazinon. The concentrations of the organophosphate pesticides ranged from average concentrations routinely detected in the Salinas Valley to extreme concentrations

detected in worst-case scenarios. It was determined that 100 µg enzyme per liter of runoff was the optimal dose to treat extreme concentrations, and the preferable interaction time was three hours.

Enzyme treatment using the optimal Landguard A900 dose was then added to the VTS. The high volume riser setting was used in these trials and the enzyme was introduced prior to the vegetated portion to allow for a maximum interaction time of three to six hours. Only low concentrations of the organophosphate pesticide dimethoate were measured during the three efficacy trials. Unlike previous studies on this farm, less organophosphate pesticides were used on the lettuce fields draining to the VTS during the current study. Concentrations of dimethoate were reduced by the VTS, but these trials did not provide a thorough assessment of the enzyme. Five additional trials with the enzyme were conducted in a larger drainage that received runoff from additional farms growing varied crops. Although the discharge in this drainage was up to twenty times greater than that of the VTS, and the mixing times were as low as 40 minutes, toxic concentrations of chlorpyrifos were reduced to below reporting or detection limits with the addition of Landguard.

As part of the reporting component of this project, four outreach presentations were given to local growers, resource agencies and regulators. These meetings were planned in conjunction with agriculture industry representatives and included an on-farm demonstration with growers, two University of California Cooperative Extension workshops, and a presentation of study results at the California Department of Pesticide Regulation. An additional set of presentations is planned for after the contract period ends. These presentations will take place as part of a special symposium at the 2012 national meeting of the Society of Environmental Toxicology and Chemistry.

## Introduction

The agricultural fields of Monterey County, California, also known as “the salad bowl of the world”, produce over \$1.4 billion of lettuce products (<http://montereycfb.com>, 2010). The \$4 billion/year agricultural industry also produces much of the nation’s strawberries, crucifer crops and grapes. Runoff from irrigated agriculture contributes a significant amount of water to local stream flow, and several studies have measured significant pesticide concentrations and biological impacts in the Salinas River (Anderson et al., 2003b; Hunt et al., 2003; Phillips et al., 2004). Pesticides in runoff from irrigated vegetable production can also impact invertebrate communities (Anderson et al., 2003a; Liess and von der Ohe, 2005; Anderson et al., 2006b), have adverse impacts on salmonids (Scholz et al., 2000), and lead to food web magnification in fish and wildlife (Pereira et al., 1996). Results of studies conducted throughout California have demonstrated that surface water toxicity is primarily caused by organophosphate and pyrethroid pesticides (Anderson et al., 2011)

While receiving system water quality would be improved by reducing applications of toxic compounds, large quantities of organophosphate and pyrethroid pesticides continue to be required on most irrigated vegetable production acreage in the Salinas Valley. Developing effective systems for runoff treatment is necessary to reduce pesticide loading to aquatic habitats. Water quality impacts and pending regulations in California have motivated growers to implement management practices to reduce pesticides and toxicity in runoff. Growers have worked with a number of agencies to design and construct vegetated treatment systems (VTS) at several locations throughout Central California (Hunt et al., 2008; Moore et al., 2008).

The integrated VTS in the current study was constructed on a working farm in a narrow linear ditch. The treatment system consisted of a short sediment settling basin followed by a vegetated portion. This VTS was previously evaluated with an additional treatment phase: addition of the enzyme Landguard™ OP-A ([www.csiro.au/solutions/pesticidebioremediation.html](http://www.csiro.au/solutions/pesticidebioremediation.html)) to break down more soluble organophosphate pesticides that were not removed with the settled sediment or by the vegetation (Anderson et al., 2011). The results of this study indicated minimal removal of diazinon by sedimentation and vegetation, but complete removal with the Landguard enzyme. The first two stages of the VTS removed greater than 90% of organochlorine pesticides in water, and up to 100% of pyrethroids in water. Most of these reductions occurred in the sedimentation portion of the VTS (Anderson et al., 2011).

Although this integrated VTS was successful at significantly reducing concentrations of organophosphate, organochlorine, and pyrethroid pesticides, toxic concentrations of pyrethroids accumulated in sediment at the end of the VTS. This was likely due to incomplete removal of pyrethroids associated with very fine-grained particles suspended in the run-off. In addition, the original Landguard dosing system, consisting of a flow-weighted valve, enzyme reservoir, battery, and small computer control unit was somewhat cost-prohibitive for routine use. Also, the previous placement of the dosing system did not retain enzyme-treated water in the vegetated portion of the system. Improvements to address these limitations were the basis for the current project.

The current project was designed to fine tune the technical specifications for conservation practices that reduce pesticide and nutrient concentrations in tailwater runoff. The first goal of the project was to refine the VTS design by measuring the performance of the vegetated component under low volume (shorter residence time) and high volume (longer residence time) scenarios. The second goal of the project was to determine the optimal dose and mixing time of the Landguard enzyme to completely detoxify representative organophosphate pesticides. This phase of the project was addressed using laboratory spiking experiments. The optimal Landguard dose was then used in the refined VTS. Lastly, Landguard efficacy was tested under conditions with high discharge and short mixing times in a larger drainage receiving agricultural runoff. The latter phase of the project was included to demonstrate the efficiency of pesticide removal in a high loading-high runoff scenario. All field trials were conducted in the VTS and larger drainage ditch during the growing season, using actual irrigation runoff events.

As part of the reporting component of this project, four outreach presentations were given to local growers, resource agencies and regulators. These meetings were planned in conjunction with agriculture industry representatives and included an on-farm demonstration with growers, two University of California Cooperative Extension workshops, and a presentation of study results at the California Department of Pesticide Regulation. An additional set of presentations is planned for after the contract period ends. These presentations will take place as part of a special symposium at the 2012 national meeting of the Society of Environmental Toxicology and Chemistry.

## **Methods**

### ***Field Study Area***

The on-farm treatment system was installed on an existing drainage ditch that was planted with vegetation by the grower with design assistance from the Resource Conservation District of Monterey County (RCD) 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, and the California Department of Pesticide Regulation (DPR).

The VTS consisted of a 260m long V-shaped ditch draining approximately 120 acres planted in row crops, primarily lettuce, broccoli, and asparagus (Figure 1). The height of the ditch was approximately one meter, the top width was 3.25m, and the bottom width was 1.25m. The ditch was fitted with an adjustable V-notched galvanized aluminum weir at Station B and an adjustable V-notched polyethylene box at Station C. The weir (Station B) was installed 40m downstream from the tailwater input (Station A), and the box (Station C) was installed 170m downstream from the weir. The ditch continued below the box for another 50m before the final exit. The fall between the input and the final exit of the ditch was approximately 35 cm. The section of the ditch between Stations A and B was not planted with vegetation, but functioned as a sediment settling basin during the trials, although some native grasses began to grow in this part of the system during the trials. The section of the ditch between Stations B and C was the vegetated section of the VTS system, and this section was previously planted with rushes

(*Juncus phaeocephalus* and *J. patens*), pennywort (*Hydrocotyle spp.*), and was seeded with creeping wild rye and red fescue. By the end of the previous study, resident Bermuda grass also flourished in the ditch. At the beginning of the current study most of the rushes had died back, but there were significant clumps that covered about 5-10% of the ditch. Bermuda grass was growing in the remainder of the ditch, but was cut back by the grower prior to the beginning of the study. Pennywort was added to the ditch before the experiments, and was flourishing within a few days. All transit time trials and Landguard trials were conducted with 100% coverage in the vegetated portion of the ditch.

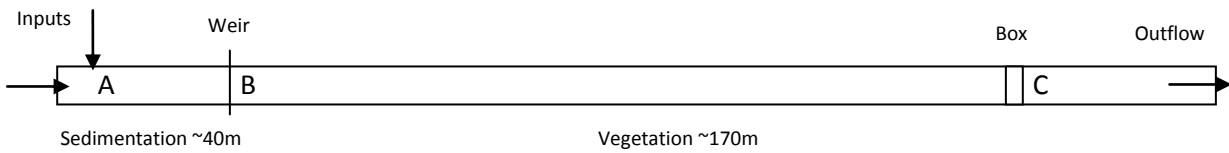


Figure 1. Schematic diagram of ditch vegetated treatment system and sampling stations (A, B, and C). Not to scale.



Figure 2. Integrated vegetated treatment system: sedimentation section and weir (left) and vegetated area (right).

### ***Low and High-Volume Trials in the VTS***

In six trials water samples were collected at Stations A, B and C of the integrated VTS to evaluate reductions in pesticide concentrations and associated toxicity based on the physical treatment effects of the system. Two trials were conducted with the box riser setting at the low position to retain a minimal amount of water in the vegetated portion of the VTS (approximately 24,000 L). Four trials were conducted with the high riser setting to retain approximately 45,000 L in the vegetated section. Because the fall between the input and the exit of the ditch was minimal, the difference between the low and high setting was only about 8 cm, but increasing the riser height nearly doubled the volume of water in the VTS.

Prior to the initiation of the trials, salinity was used as a conservative tracer to determine the pulse transit time (PTT) of water moving through the VTS. PTTs were calculated to determine the amount of time it would take for water to move through the system based on the magnitude and duration of the input flow. This information was used to determine the amount of time a pulse of water spent in the vegetated portion of the VTS, and the appropriate time to collect downstream samples so that the treated pulse of water could be captured and tested. PTTs were also determined for the Lateral Ditch and this information was used to determine enzyme mixing times in the Landguard application trials.

Water level loggers were placed in protective housings approximately one meter upstream of both the weir and the box to record water height, conductivity and temperature. The housings consisted of one-inch PVC with drilled holes every few cm, and were securely fastened to a metal fence stake. Natural seawater (1000 liters from MPSL) was added to the VTS immediately downstream of the weir (Station B) during five irrigation events. As irrigation water flowed over the weir, it pushed the pulse of seawater through the system, and the peak height of the water flowing over the weir was recorded by the logger. As the seawater pulse reached the box, the downstream logger recorded the increase in conductivity. We determined the travel time for a pulse of water during any given irrigation event by comparing the peak water height at the weir with the amount of time the pulse took to travel the length of the VTS. A clear salinity signal was observed during six tracer trials with the box at the lower setting, and the PTT was determined based on the magnitude of each irrigation event (Figure 2). A curve based on these irrigation events was used to predict the PTT and thus determined the sample collection times for the two trials conducted with the box at the low riser setting.



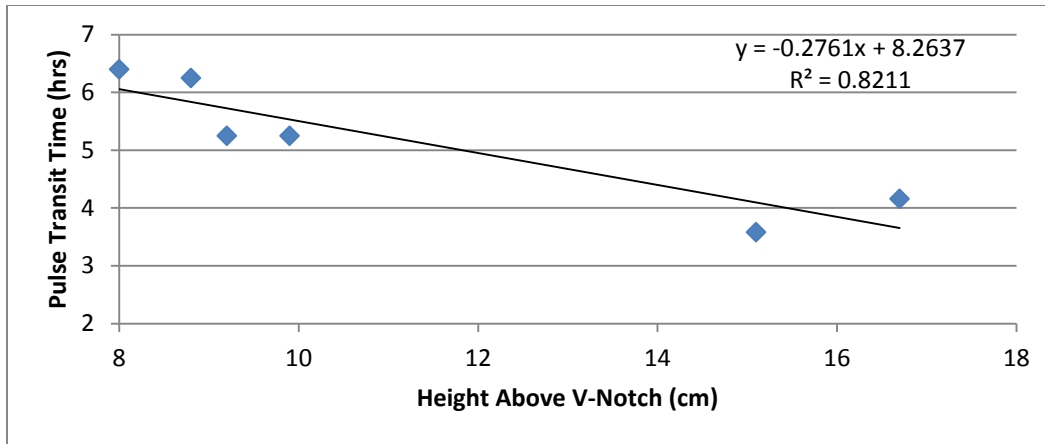


Figure 2. Pulse transit times (hours) versus discharge, as measured by the height of the water in the v-notch of the weir.

An initial input sample (Station A) was collected at the beginning of each irrigation event. Water was allowed to pass through the settling basin for one hour before three weir samples (Station B) were collected fifteen minutes apart. Samples were spaced 15 minutes to bracket the presumed PTT for the section of the system between Stations A and B. These samples were composited in the laboratory prior to testing and analysis. Box samples (Station C) were collected at specific times based on the peak flow of the water over the weir. Three box samples bracketing the modeled PTT between Stations B and C were collected at fifteen minute intervals and composited at the laboratory, prior to testing and chemical analysis.

Based on the salinity trials at the higher riser setting on the box, the PTT was dependent on the overall input water volume during the monitoring of the pulse, and not on the peak flow. PTTs during the salinity trials ranged from three to six hours with the salinity signal being greatly diluted by the time it reached the box. Because the higher box setting withheld almost twice the volume of water in the VTS as the lower setting, the pulse moving through the box was spread out over a longer period. In the four trials at the higher setting, the input sample was collected upon arrival, and the weir samples were collected one hour later at 15 minute increments (at 60, 75 and 90 minutes). The box samples were collected 3, 4.5 and 6 hours after the initial weir sample. Because of the higher variability of the PTTs at the higher riser setting, the three samples were spread over a longer period to bracket the pulse.

All of the trials included water toxicity tests, total suspended solids analysis, and ELISA (enzyme-linked immunosorbent assays for diazinon and chlorpyrifos) conducted on samples collected from the input, weir and box. Samples from four of the trials were also analyzed for organophosphate (EPA Method 8141), organochlorine (EPA Method 8081), and pyrethroid pesticides (EPA Method 1660) using gas chromatography with confirmation by mass spectroscopy (GC/MS).

### ***Lateral Ditch Hydrology***

The VTS ditch drains into a larger lateral ditch that conveys runoff from five additional farms. Experiments were conducted in this ditch to determine the effectiveness of Landguard in higher flow regimes with shorter mixing times. The lateral ditch is approximately 300m long and falls approximately 2m from the weir to the end of the study area. Although there is crab grass growing in the banks, the bottom of the ditch is severely incised and un-vegetated. The average discharge over the weir in the VTS was 0.9 L/s, whereas the average discharge over the weir in the lateral ditch is 18 L/s. Because of the higher flow rate and sharp fall, water can pass through the lateral ditch study reach in as little as ten minutes.

The PTT of water moving through the lateral ditch was modeled using the same method described above. The PTT data were used in the Landguard application trials described below. A weir was constructed on the upper end of the lateral ditch, immediately downstream of the input from the tailwater ponds described in Hunt et al. (2008), and a logger housing was installed about one meter upstream of the weir. The lateral ditch weir had a 90-degree v-notch. There was not a structure on the lower end of the lateral ditch, but a logger housing was installed upstream of the lower culvert. Seawater was added immediately below the weir during irrigation events. As previously described, the upper logger recorded the peak height of the water going over the weir and the lower logger recorded the increase in conductivity as the pulse of seawater reached the bottom of the ditch.

Seawater was added to the lateral ditch during five trials. Whereas the rate of water passing over the VTS weir was approximately 1-2 L/s, the rate of the water passing over the lateral weir averaged about 13 L/s but ran as high as nearly 40 L/s. Because of the greater volume of water traveling through the lateral ditch, the PTTs were much shorter. The height of the water in the v-notch of the weir ranged from approximately 8 to 21 cm and the PTTs ranged from 10 to 50 minutes.

### ***Landguard™ A900 Enzyme***

Landguard A900 enzyme was developed by the Commonwealth Scientific and Industrial Research Organization (CSIRO), Australia's national science agency. The enzyme is derived from bacteria originally isolated from soil and cultured on a large scale to produce commercial quantities of enzyme for use in treatment of agriculture runoff, contaminated soils, animal pest treatment wastewater, and other applications where rapid treatment is needed to break down organophosphate pesticides to non-toxic metabolites. The final product for use in the system described below was produced using the following steps (Craig Clarke, ORICA Watercare, personal communication): 1) Fermentation of the bacteria that produces the enzyme. 2) Lysing (homogenization) the bacterial cells to release the enzyme from within the cell. 3) Flocculation, to remove a significant proportion of the cell debris (cell walls etc). 4) Centrifugation of the ferment media to remove more of the cell debris. 5) Passing the lysed ferment media through a number of filtration steps (including sterile filtration) to sterilize the ferment media and remove further cell debris. 6. The liquid product is then freeze-dried and packaged. There are no chemicals added to the freeze dried ferment media. The Landguard product is made up of the active

A900 enzyme and bacterial cell debris (largely carbohydrates, water and proteins). The A900 enzyme acts as a catalyst for the rapid hydrolysis of pesticides, producing non-toxic metabolites.

### ***Laboratory Efficacy Experiments***

In a previous field study with the precursor of Landguard A900, Landguard OP-A, the enzyme was applied at a concentration of 100 µg/L of water (Anderson et al., 2011). According to the manufacturer's recommendations, this appeared to be the optimal concentration to treat the expected concentrations of organophosphates in furrow runoff. In the current study, three laboratory efficacy experiments were conducted where three concentrations of Landguard were combined with three concentrations of chlorpyrifos or diazinon over 3 different mixing times.

Chlorpyrifos and diazinon stock solutions (100 µg/mL) were purchased from Accustandard (New Haven, CT). Stock solutions were used to prepare test concentrations of 50, 500, and 5,000 ng/L for chlorpyrifos and 100, 1,000, and 10,000 ng/L for diazinon. These concentrations were chosen to bracket actual concentrations of organophosphates measured in agricultural drainages as part of previous projects. Landguard A900 stock solution was prepared at a concentration of 10 mg/L. The stock solution was used to prepare treatment concentrations of 1, 10, and 100 µg/L. Each Landguard concentration was combined with each organophosphate concentration. The Landguard and pesticides were allowed to mix for 1 hour and 3 hours to determine the effect of mixing time on pesticide hydrolysis.

The concentrations of organophosphates in the baseline and treatments were confirmed using enzyme-linked immunosorbent assays (ELISA, Strategic Diagnostics Inc, Newark, DE). ELISA procedures followed those recommended by Sullivan and Goh (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. Precision was determined by duplicate measurement of one sample per batch. Samples were tested without dilution unless necessary. Lowest detectable concentrations for this procedure were 30 ng/L for diazinon and 50 ng/L for chlorpyrifos. Reporting limits were twice the lowest detectable concentrations. One set of baseline concentrations was also confirmed with GC/MS (EPA Method 8141).

### ***Landguard Field Application Trials***

Landguard was applied to water flowing through the VTS and the lateral ditch at the respective upstream weirs. A stock solution of 1000 mg/L was prepared in a plastic carboy in the field. The stock solution was delivered to the weir via silicone tubing running through a positive pressure pump powered by a portable generator. The pump rates were controlled manually based on the height of the water as measured by the staff plate. The final concentration of Landguard in the water was based on results of the laboratory experiments which showed the optimal treatment concentration was 100 µg/L.

Three Landguard applications were conducted in the VTS with the box at Station C adjusted to the high setting for maximum volume in the vegetated treatment section of the ditch. Each Landguard application was conducted during a single irrigation event during summer 2011. A baseline sample was collected upstream of the weir at the beginning of the application. Downstream samples were collected at the box based on the methods described above (3, 4.5 and 6 hours after the beginning of application). Five Landguard applications were conducted in the lateral ditch on separate irrigation events during summer 2011. Samples in the lateral ditch were collected at the downstream station based on the PTT calculations described above. This station was approximately 300 meters downstream of the weir. All of the trials included water toxicity tests with *C. dubia* and *H. azteca*, total suspended solids analysis, and ELISA conducted on water samples collected from the weirs and downstream stations. Two of the VTS trials and three of the lateral ditch trials also included analysis of water samples for organophosphate (EPA Method 8141) and pyrethroid pesticides (EPA Method 1660) with GC/MS.

### **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. Surficial sediments were collected using a polycarbonate core tube and placed into 2-liter glass jars. Sample containers were immediately placed in coolers with sufficient wet ice to adjust and maintain the temperature at  $4 \pm 3^\circ \text{C}$  during transport to MPSL. Water samples were stored at  $4 \pm 3^\circ \text{C}$  for no longer than 48 hours prior to toxicity test initiation. After a minimum of 16 hours in storage, bottles were inverted several times to re-suspend the settled particles. 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 4-day acute toxicity tests with the cladoceran *Ceriodaphnia dubia* and the amphipod *Hyalella azteca*, a resident species (USEPA, 2002). Each undiluted sample was tested using five replicates containing five *C. dubia* neonates or ten *H. azteca*. Neonates were <24 hours old and were obtained from Toxscan (Watsonville, CA) or from in house cultures. Amphipods were 7-14 days old and were obtained from Chesapeake Cultures (Hayes, VA). Survival was monitored daily. Daphnid tests were fed algae and YCT (yeast, cerophyll, and trout chow) daily two hours prior to renewal. Amphipod were fed YCT and renewed at 48 hours. Sediment toxicity was assessed using the 10-day growth and survival toxicity test with *H. azteca* (USEPA, 2000). Each sample was divided among eight laboratory replicates containing approximately 100 mL of sediment and 175 mL of overlying water. Each replicate was tested with ten amphipods that were 7-14 days old. The test temperature for *C. dubia* was  $25 \pm 1^\circ \text{C}$  and the test temperatures for *H. azteca* tests were  $25 \pm 1^\circ \text{C}$ . Overlying water was renewed twice daily, and 1.5 mL YCT 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. Water quality parameters measured in all tests included dissolved oxygen, pH, conductivity, ammonia, hardness, and alkalinity measurements. Nitrate, phosphate and turbidity were measured in the water samples. The response of test organisms was evaluated using the Test of Significant Toxicity (USEPA, 2010). All concentrations of pesticides measured in water and sediment were compared to published toxicity thresholds. The median lethal concentrations (LC50s) used for comparison to the water chemistry data are provided in Table 1.

Table 1. *Ceriodaphnia dubia* water median lethal concentrations, and *Hyalella azteca* water and sediment median lethal concentrations (LC50s).

Chemical	ng/L	Days	Endpoint	Reference
<i>Ceriodaphnia dubia</i>				
Cyhalothrin	200	2	LC50	(Wheelock et al., 2004)
Permethrin	250	2	LC50	(Wheelock et al., 2004)
Chlorpyrifos	54	4	LC50	(Bailey et al., 1997)
Diazinon	320	4	LC50	(Bailey et al., 1997)
Malathion	2120	2	LC50	(Ankley et al., 1991)
<i>Hyalella azteca</i> (water)				
Cyhalothrin	2.3	2	EC50	(Maund et al., 1998)
Permethrin	21.1	4	LC50	(Anderson et al., 2006a)
Chlorpyrifos	86	10	LC50	(Phipps et al., 1995)
Diazinon	6510	4	LC50	(Ankley and Collyard, 1995)
pp DDT	70	10	LC50	(Phipps et al., 1995)
<i>Hyalella azteca</i> (sediment)				
	ng/g	µg/g oc		
Bifenthrin	12.9	0.52	10	LC50 (Amweg et al., 2005)
Cyhalothrin	5.6	0.45	10	LC50 (Amweg et al., 2005)
Cypermethrin	14.9	0.38	10	LC50 (Maund et al., 2002)
Esfenvalerate	41.8	1.54	10	LC50 (Amweg et al., 2005)
Permethrin	201	10.8	10	LC50 (Amweg et al., 2005)
Chlorpyrifos		1.77	10	LC50 (Amweg and Weston, 2007)

## Results and Discussion

### Quality Assurance

All toxicity test controls had acceptable survival (>90%) based on the criteria set forth in the U.S. EPA protocols. Toxicity testing precision was evaluated with reference toxicant tests and with field duplicates. Current reference toxicant tests were evaluated in relation to past test performance. Reference toxicant tests were conducted using the standard protocol on a dilution series of copper for *C. dubia* and cadmium for *H. azteca*. Both the *C. dubia* and *H. azteca* responses, measured as LC50s, were within the control chart confidence limits (Figure 3), indicating that test organisms responded to the toxicant in a manner consistent with previous tests.

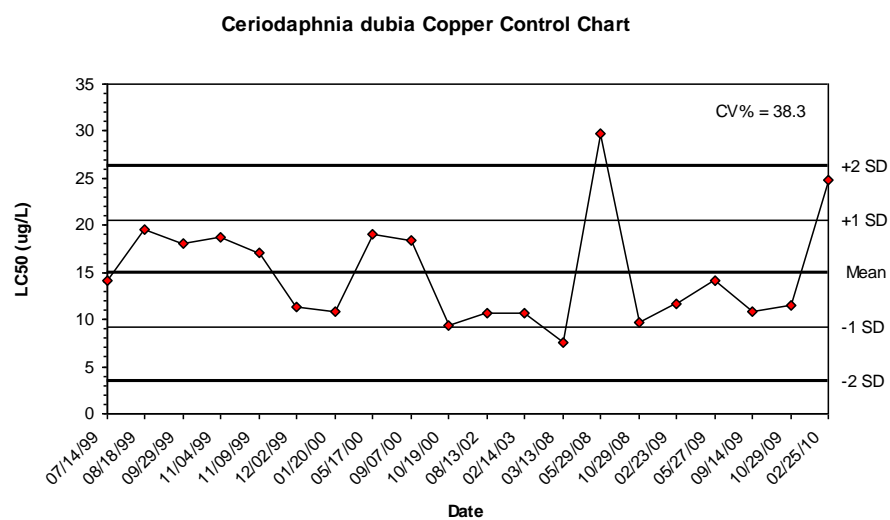


Figure 3a. *C. dubia* reference toxicant control chart.

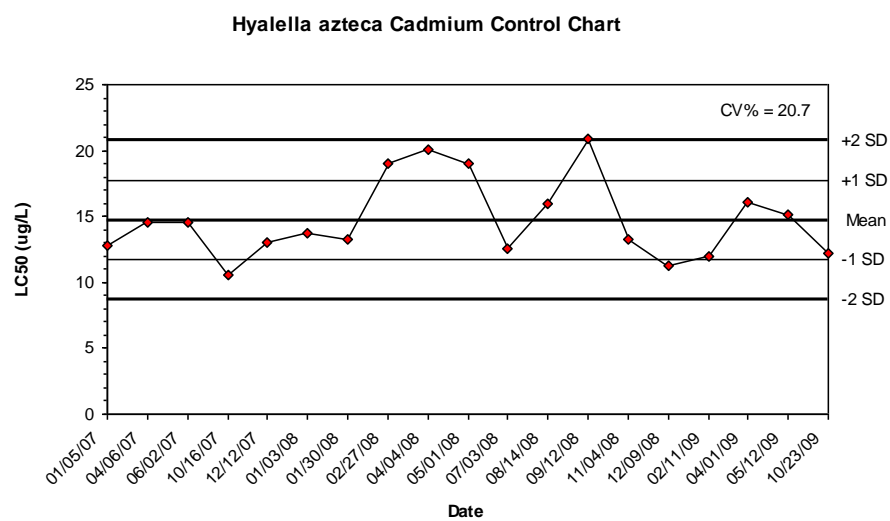


Figure 3b. *H. azteca* reference toxicant control chart.

ELISA chemistry precision and accuracy were evaluated through the analysis of external laboratory reference materials and the measurement of sample duplicates. ELISA reference material recoveries ranged from 114% to 143% for chlorpyrifos and 122% to 164% for diazinon. All sample duplicates were below reporting limits. GC/MS chemistry precision and accuracy were evaluated through the analysis of external laboratory reference materials, blank spikes, and the measurement of sample duplicates. Reference material recovery ranged from 50-125%. GC/MS reference material recoveries in deionized water were all within acceptable ranges. Recoveries of pyrethroids, organophosphates and organochlorines ranged from 55-150%. Recoveries in sediment ranged from 66-117%. Relative percent differences between GC/MS duplicates were 28% or less.

#### ***Low and High-Volume Trials in the VTS (Toxicity and Chemistry)***

The volumes of water entering the VTS during the sample collection period of the two low-volume trials were 11,000L and 41,000L. The volumes during the sample collection period of the high-volume trials ranged from 26,000L to 71,000L. These volumes were generally lower than the volumes measured by Anderson et al. (2011), which ranged from 38,000L to 113,000L. Percent infiltration was measured in the high volume trials by comparing input and output volumes. The average infiltration on the high volume trials was 42%. Anderson et al. (2011) measured an average of 48% infiltration.

The TSS in the water entering the ditch at the input ranged from 89 to 3890 mg/L, but was reduced to approximately 36 mg/L before passing over the weir (Table 2). Total suspended solids were further reduced in the vegetated portion of the ditch to approximately 17 mg/L. Final suspended solids concentrations as measured by turbidity were less than 17 NTU. No organophosphate pesticides were detected with ELISA in either trial.

Water samples in the second trial were analyzed for pesticides and low concentrations of organophosphates and organochlorines were detected. Pyrethroid pesticides were not detected, but the reporting limits for this trial were 15 ng/L, so some pyrethroids might have been present at concentrations below the reporting limit. Low concentrations (below toxicity thresholds of the organisms) of diazinon and trace amounts of malathion were detected at all points along the VTS, and were not reduced by the system. Although the TSS was greatly reduced by the VTS, low concentrations of organochlorines were only reduced by 32% in the second trial (Table 2).

Table 2. Mean organism survival (standard deviation) and concentrations of detected constituents in water samples. Shading indicates significant toxicity or chemical concentrations exceeding the organism LC50. ND indicates non-detectable concentration.

9/9/2010	<i>C. dubia</i> (% Survival)		<i>H. azteca</i> (% Survival)		TSS (mg/L)	Pyrethroids (ng/L)		Organophosphates (ng/L)			Organochlorines (ng/L)						Nutrients (mg/L)	
	Mean	SD	Mean	SD		CYH	PER	CHL	DIA	MAL	ΣDDT	4,4'-DDT	ΣCHLOR	Dacthal	Dieldrin	Toxaph.	Nitrate	Phosph.
A-Input	92	11	0	0	3890	NA	NA	<RL	<RL	NA	NA	NA	NA	NA	NA	NA	18.9	4.45
B-Weir	0	0	0	0	32.0	NA	NA	<RL	<RL	NA	NA	NA	NA	NA	NA	NA	25.6	6.14
C-Box	0	0	20	10	17.5	NA	NA	<RL	<RL	NA	NA	NA	NA	NA	NA	NA	13.5	3.37
Control	96	9	90	10				ELISA	ELISA									
9/17/2010																		
A-Input	80	20	80	10	89.0	ND	ND	ND	26	Trace	60.5	6.48	0	34.3	ND	226	16.9	1.97
B-Weir	44	17	80	10	39.0	ND	ND	ND	24	Trace	35.4	3.27	0	33.6	ND	256	15.9	1.65
C-Box	65	17	93	66	17.0	ND	ND	18	29	Trace	15.8	2.51	0	29.1	ND	172	17.9	2.58
Control	92	11	90	0														
5/4/2011																		
A-Input	92	11	20	23	570	5.03	26.9	ND	ND	ND	321	119	0	ND	ND	ND	25.4	7.95
B-Weir	100	0	48	15	85.5	ND	39.2	ND	ND	Trace	84.5	43.9	0	ND	ND	ND	38.4	3.37
C-Box	97	7	72	8	11.6	ND	25.1	12	12	80	10.6	ND	0	ND	ND	ND	36.4	3.86
Control	92	11	98	4														
5/6/2011																		
A-Input	47	30	2	4	1340	NA	NA	ND	ND	NA	NA	NA	NA	NA	NA	NA	38.5	3.01
B-Weir	93	15	24	22	99.3	NA	NA	ND	ND	NA	NA	NA	NA	NA	NA	NA	30.8	3.05
C-Box	87	18	96	5	20.3	NA	NA	ND	ND	NA	NA	NA	NA	NA	NA	NA	23.1	5.35
Control	93	15	94	5				ELISA	ELISA									
5/11/2011																		
A-Input	0	0	0	0	3764	33.5	68.1	12	ND	ND	575	109	44.3	ND	ND	ND	17.1	1.92
B-Weir	88	18	28	8	234	11.1	18.1	ND	ND	ND	98.7	42.3	0	ND	ND	ND	19.1	1.56
C-Box	88	11	94	5	30	ND	ND	11	ND	ND	13.8	ND	0	ND	ND	ND	19.7	2.06
Control	88	18	100	0														
5/12/2011																		
A-Input	52	30	6	13	3164	123	107	ND	ND	ND	522	85	17.6	ND	8.3	ND	23.9	2.12
B-Weir	92	11	18	11	74.7	27.8	13.1	ND	18	Trace	45.5	15.6	0	ND	ND	ND	21.1	2.24
C-Box	96	9	62	15	15.7	5.71	ND	ND	17	Trace	0	ND	0	ND	ND	ND	20.3	2.23
Control	92	11	94	9														
<i>C. dubia</i> LC50						200	250	53	320	2120								
<i>H. azteca</i> E/LC50						2.3	21.1	86	6510		70							



The input sample in the first trial was toxic to *H. azteca*, but not *C. dubia* (Table 2). This indicates that pyrethroids might have been present because *H. azteca* are more sensitive to this class of pesticides. Toxicity to *H. azteca* was slightly reduced by the vegetated portion of the ditch, but toxicity to *C. dubia* increased. Although no chlorpyrifos or diazinon was detected and pyrethroids were not measured, it is apparent that the water already present in the VTS contained an elevated concentration of some contaminant. Based on the low input of water relative to other events (11,000L), it is apparent that the vegetated portion of the ditch, which contains approximately 23,000L, was inadequately flushed. Water retention appears to have also occurred in the second trial because toxicity to *C. dubia* increased in the lower portions of the VTS. No toxicity to *H. azteca* was observed in the second trial. The water infiltration rate was not measured in the first trial, but in the second trial there was no infiltration, indicating that there was already water present in the ditch. No organophosphates or organochlorines greater than toxicity thresholds were measured in the second trial, but the 15 ng/L reporting limit for pyrethroids was above reported toxicity thresholds. It is unclear what contributed to the toxicity of *C. dubia* in the second trial. The pyrethroid analytical method was improved for subsequent trials and the pyrethroid reporting limit for these trials was reduced to 5 ng/L.

Significant toxicity to at least one of the test organisms was observed in all four high-volume trials (Table 2). As the pulse of water moved through the VTS, toxicity was reduced in all cases. ELISA did not detect chlorpyrifos or diazinon, but GC/MS analysis detected low concentrations of three organophosphates. None of these organophosphate concentrations were greater than the toxicity thresholds. The pyrethroid pesticides cyhalothrin and permethrin were detected at concentrations greater than organism LC50s in all three high-volume trials that included GC/MS analysis. Potentially toxic concentrations of 4,4' DDT were also detected in these trials. Concentrations of these chemical classes were reduced by 97-100% in all trials except one. Permethrin concentrations were not reduced in the 5/4/2011 trial. In all other trials the reduction of these hydrophobic pesticides correlates with the reduction of TSS, which was reduced by 98-100%. Final suspended solids concentrations as measured by turbidity ranged from 41-103 NTU. Reduction of these pesticides also correlated significantly with a decrease in amphipod mortality (Figure 4). The pesticides detected at toxic concentrations were cyhalothrin, permethrin and 4,4' DDT, but the majority of the contribution to the toxic units came from the two pyrethroids.

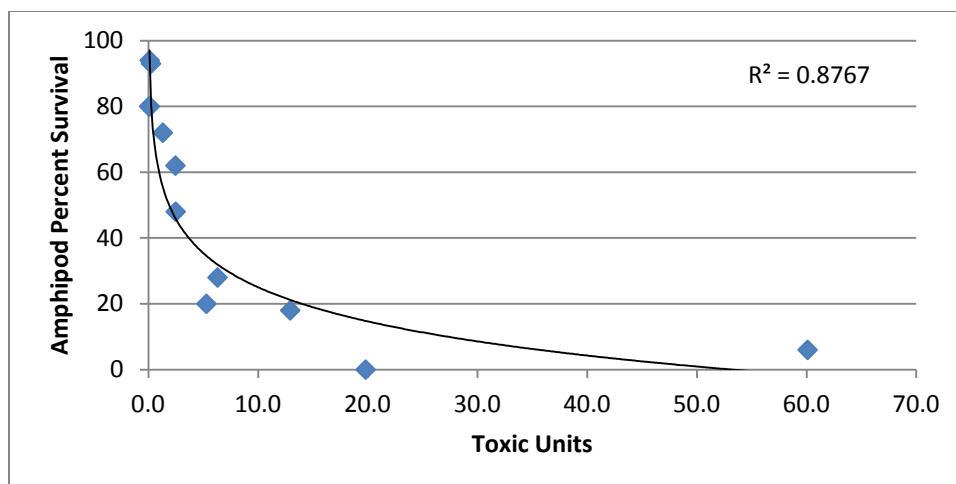


Figure 4. The relationship between percent amphipod survival and the sum of pesticide toxic units (calculated from water LC50s of cyhalothrin, permethrin, chlorpyrifos, diazinon, and three DDT metabolites).

Nitrate concentrations measured at the input ranged from 17 mg/L to 39 mg/L, and phosphate concentrations ranged from 2 mg/L to 8 mg/L (Table 2). Concentrations measured at the box were only slightly lower with ranges of 14 mg/L to 36 mg/L for nitrate and 2 mg/L to 5 mg/L for phosphate. Although the average concentrations were slightly lower at the box, significant amount of these nutrients are not being removed by the VTS.

### ***Sediment Toxicity and Chemistry***

Sediment toxicity at the weir and box was measured during one trial to determine if the particles depositing at the lower end of the system were less toxic than those falling out of the water column at the upper part of the system. Significant toxicity was observed in sediment from both the weir and box stations (Table 3). The concentration of chlorpyrifos in the sediment collected at the weir was high enough to cause the observed toxicity, whereas the sediment from the box contained toxic concentrations of cyhalothrin and cypermethrin. Toxic units for each chemical can be summed by dividing the organic carbon-corrected chemical concentration by the corresponding LC50 (from Table 1). Assuming additivity, the toxic units of each pyrethroid can be summed with those of chlorpyrifos. The total toxic unit values measured in the sediment at the weir and box were 1.8 and 4.6, respectively. Based on the chemistry results, there are enough pyrethroids associated with the fine particles that are moving through the system to account for the observed toxicity. These results are similar to those reported by Anderson et al. (2011), and indicate that fine-grained particles that traverse the sedimentation and vegetated sections of the ditch accumulate at toxic concentrations at the bottom of the system. Sediment-bound pyrethroid pesticides have half-lives on an order of weeks to months depending on the chemical. The optimal situation is to completely remove particles from the water column, retain the particles in the VTS, and allow pyrethroids to degrade. Given that degradation rates for certain pyrethroids take several months to over a year (e.g., bifenthrin), management of

contaminated sediments within farm ditches may require occasional dredging and drying of accumulated sediment to facilitate photolytic breakdown. An alternative would be to use newly developed synthetic pyrethroid enzymes to detoxify residual pyrethroids accumulated at the base of the treatment system. The efficacy of these enzymes is the subject of future studies.

Table 3. Mean percent survival (standard deviation) of *Hyalella azteca* exposed to sediment from weir and box, and concentrations of pesticides detected in sediments.

		B-Weir		C-Box		Control
<i>H. azteca</i>	Mean % Surv. (SD)	0 (0)		3 (5)		94 (7)
		ng/g	ug/g oc	ng/g	ug/g oc	
Pyrethroids	Bifenthrin	ND	ND	4.95	0.454	
	Cyhalothrin	1.31	0.076	8.66	0.794	
	Cypermethrin	ND	ND	6.1	0.560	
	Es/Fenvalerate	2.01	0.116	ND	ND	
	Fenpropathrin	ND	ND	1.17	0.109	
	Permethrin	13.18	0.762	29.50	2.71	
Organophosphates	Chlorpyrifos	44.3	2.56	4.84	0.444	
Organochlorines	2,4'-DDD	1.7	0.098	1	0.092	
	2,4'-DDT	1.4	0.081	1.4	0.128	
	4,4'-DDD	4.3	0.249	2.2	0.202	
	4,4'-DDE	41.3	2.39	30.7	2.82	
	4,4'-DDT	2.8	0.162	3.3	0.303	

### Laboratory Efficacy Experiments

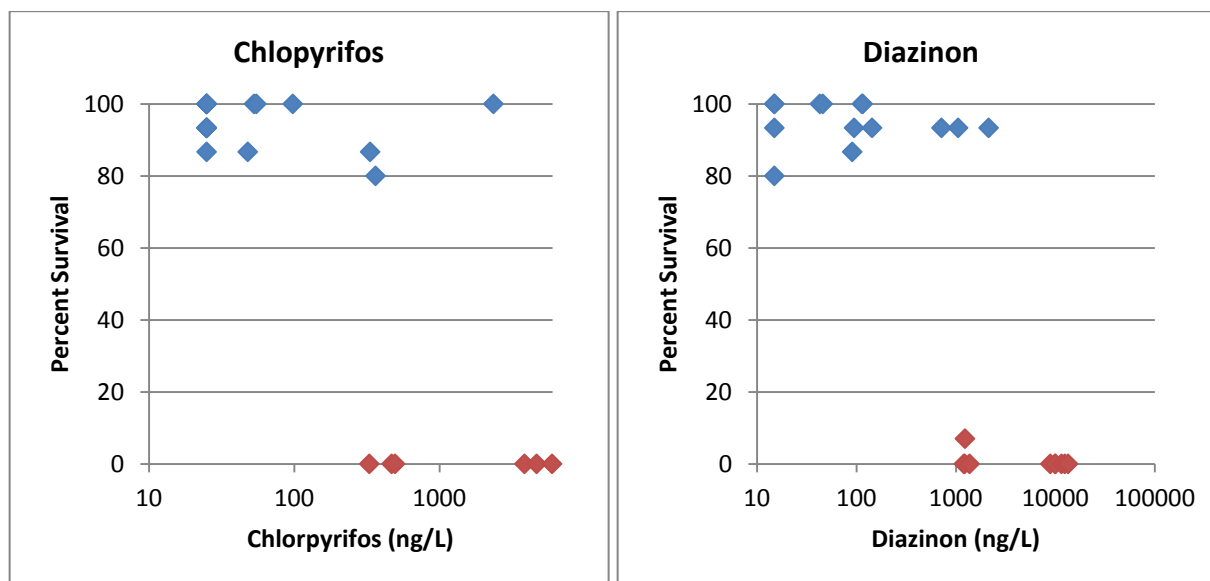
Three doses of Landguard A-900 were added to three separate concentrations of chlorpyrifos and diazinon, and allowed to mix for one and three hours. The resulting 3x3x2 test matrix was conducted in triplicate for each pesticide. Pesticide concentrations of the lab trials were measured with ELISA, and the nominal concentrations in the third trial were confirmed with GC/MS. According to the ELISA results, the measured concentrations ranged from 97 to 107% of nominal for chlorpyrifos and 129 to 135% of nominal for diazinon. Samples measured by GC/MS had lower recoveries. Chlorpyrifos concentrations ranged from 48 to 80% of nominal, and diazinon concentrations ranged from 59 to 84% of nominal. ELISA analysis was conducted on the same day of the experiments, whereas the GC/MS analysis was conducted several days later, but within the acceptable holding time of seven days (SWAMP, 2008). Studies by the Department of Pesticide Regulation demonstrate that organophosphates can break down by as much as 35% during a seven-day holding time (Sue Peoples, DPR Sacramento, personal communication). The loss in the current samples was as high as 52%. Based on these results, the laboratory experiments were evaluated based on concentrations measured by ELISA.

A previous study with Landguard OP-A in the VTS and a nearby settling pond utilized an enzyme dose of 100 ug/L (Anderson et al., 2011). Treated concentrations of chlorpyrifos and diazinon in these systems were as high as 150 ng/L and 2620 ng/L, respectively, but diazinon has been measured at over 15,000 ng/L. Mixing times in this study were estimated at approximately one hour for the VTS and on the order of days for the pond system. In the current study, concentrations of chlorpyrifos as high as 5000 ng/L were adequately treated by 100 ug/L Landguard (Table 4). The 500 ng/L concentration was not completely hydrolyzed at Landguard concentrations less than 100 ug/L, even when allowed to mix for three hours. Almost all concentrations of diazinon were broken down by the 100 ug/L enzyme treatment when allowed to mix for three hours. The lower Landguard doses did not reduce the concentrations of diazinon below the *C. dubia* LC50 value of 320 ng/L (Bailey et al., 1997).

The third laboratory trial of enzyme treatment of pesticides also included toxicity exposures with *C. dubia* to verify no residual toxicity remained after treatment with Landguard. No toxicity was observed in the lowest baseline treatments of chlorpyrifos and diazinon, but toxicity was observed in the upper two baseline treatments that were not treated with Landguard (Figure 5). All toxicity was removed at the highest enzyme treatment after three hours. In several cases measured concentrations of organophosphates were well above the *C. dubia* LC50s, but no toxicity was observed. This occurred four times with chlorpyrifos, at concentrations of 98, 334, 364 and 2360 ng/L, and twice with diazinon at concentrations of 1056 and 2140 ng/L. These situations generally occurred at the lower Landguard treatments and at the one-hour mixing time, and occurred randomly. There were a number of situations where the concentration of organophosphate was higher than those listed above and toxicity was observed (Figure 5). It is possible that the bioavailability of the organophosphate was reduced through binding to the enzyme treatment. If this were the case, the ELISA analysis may still have detected the bound pesticides, even though they were not bioavailable.

Table 4. Laboratory efficacy tests with Landguard A900. Four concentrations of enzyme were added to three concentrations each of chlorpyrifos and diazinon. Mixtures were allowed to interact for one or three hours. ND and shading indicate non-detects.

Nominal Chlorpyrifos	Lab Trial 1				Lab Trial 2				Lab Trial 3			
	Landguard Concentration (ug/L)				Landguard Concentration (ug/L)				Landguard Concentration (ug/L)			
	0	1	10	100	0	1	10	100	0	1	10	100
<b>One Hour</b>												
50 ng/L	ND	ND	ND	ND	ND	ND	ND	ND	48	ND	ND	ND
500 ng/L	432	376	320	ND	372	320	142	ND	470	364	98	ND
5000 ng/L	2300	2780	2280	ND	3280	2520	1620	ND	4680	3860	2360	ND
<b>Three Hour</b>												
50 ng/L	ND	ND	ND	ND	ND	ND	ND	ND	53	ND	ND	ND
500 ng/L	354	280	180	ND	370	304	ND	ND	496	334	55	ND
5000 ng/L	2940	2420	1300	ND	3320	1560	ND	ND	5980	3300	ND	ND
<b>Nominal Diazinon</b>	Landguard Concentration (ug/L)				Landguard Concentration (ug/L)				Landguard Concentration (ug/L)			
	0	1	10	100	0	1	10	100	0	1	10	100
<b>One Hour</b>												
100 ng/L	98	103	86	ND	88	74	64	ND	144	114	91	ND
1000 ng/L	894	1018	1038	500	852	682	672	122	1208	1228	1056	46
10000 ng/L	10719	10045	10000	3560	9580	9540	6360	ND	13460	12460	11540	2140
<b>Three Hour</b>												
100 ng/L	91	77	60	ND	82	81	40	ND	116	95	43	ND
1000 ng/L	814	670	578	60	912	660	510	ND	1376	1238	718	ND
10000 ng/L	7980	7640	5910	ND	11240	8800	4100	ND	10000	10000	8920	ND



## ***Landguard Field Application Trials***

### *VTs Trials*

Studies with vegetated systems have demonstrated the difficulty of reducing loads of more soluble organophosphate pesticides with vegetation (Hunt et al., 2008; Moore et al., 2008). The addition of the Landguard enzyme to these systems provides another phase of treatment in addition to sedimentation and vegetation. Landguard OP-A was effective at removing diazinon in a previous study of the VTS that is the subject of the current project (Anderson et al., 2011). This study demonstrated the need to further optimize the integrated system and to fine-tune the enzyme dosing and mixing times. In addition, a new Landguard A900 formula was evaluated to provide comparative data to the Landguard OP-A used in the original study.

In the previous VTS study, the enzyme was applied to the system after the vegetated portion of the ditch (Anderson et al., 2011). Application at that point allowed for approximately one hour of mixing time before the water exited the system. In the current study Landguard A900 was applied at the weir on the upper end of the vegetated portion of the system. Application at the weir allowed for 3-6 hours of mixing time before post-vegetation samples were collected. Based on the laboratory tests, a 100 ug/L dose was used to treat the system, and the box setting was left in the upper position to retain as much water as possible in the vegetated portion of the system.

Water samples collected during the three VTS Landguard trials only contained low or trace concentrations of the organophosphate dimethoate, which were effectively removed by the addition of the enzyme (Table 5). Pyrethroids were measured in two of the trials, and were detected at toxic concentrations in the weir samples, and at a partially toxic concentration in the first sample collected at the box. The toxicity results corroborated the chemistry results. Significant toxicity to both organisms was observed in the first trial, and tracked with the measured concentrations of cyhalothrin. Only toxicity to *H. azteca* was observed in the second trial, and also tracked with the concentration of cyhalothrin. The VTS reduced the concentration of cyhalothrin by 98% when the input water contained approximately 100 toxic units of the pyrethroid, and cyhalothrin was reduced 100% when the input contained only about 4 toxic units of the pyrethroid. Toxicity to *H. azteca* was observed in the third trial, but pyrethroid concentrations were not analyzed during this trial.

Because only small amounts of dimethoate were detected in the system, it was impossible to thoroughly evaluate the effectiveness of the Landguard treatments. At the time of the trials, the field draining to the VTS contained crops of leaf and head lettuce. The cooperating grower informed us that he had reduced his use of organophosphates on his lettuce crops, in particular diazinon. The grower continues to use chlorpyrifos on broccoli, but these fields were not draining to the VTS during the study period.

Table 5. Percent survival (standard deviation) of daphnids and amphipods, and physical and chemical measurements in water samples collected during Landguard application trials in the VTS. ELISA was used to measure chlorpyrifos and diazinon in the third trial.

5/25/2011	<i>C. dubia</i> (% Survival)		<i>H. azteca</i> (% Survival)		Organophosphates (ng/L)			Pyrethroids (ng/L)		Turbidity (NTU)	Nitrate (mg/L)	Phosphate (mg/L)
	Mean	SD	Mean	SD	CHL	DIA	DIM	CYH	PER			
B-Weir	0	0	0	0	ND	ND	85	231	2.87	52	25.1	3.37
C-Box	84	26	48	8	ND	ND	Trace	5.03	ND	51	23.6	2.55
Control	100	0	96	5								
5/26/2011												
B-Weir	84	26	13	12	ND	ND	Trace	8.2	ND	103	21.9	2.54
C-Box	96	9	91	5	ND	ND	ND	ND	ND	15	16.3	1.52
Control	88	11	100	0								
6/3/2011												
B-Weir	92	18	12	18	ND	ND	NA	NA	NA	237	50.6	4.97
C-Box	100	0	92	11	ND	ND	NA	NA	NA	31	37.2	2.59
Control	92	18	100	0								
<i>C. dubia</i> LC50					53	320		200	250			
<i>H. azteca</i> E/LC50					86	6510		2.3	21.1			

#### Lateral Ditch Trials

Discharge rates in the lateral ditch were approximately twenty times those observed in the VTS. Although the lateral ditch was only about 40 meters longer than the VTS, pulse transit times in the lateral ditch ranged from 20 to 45 minutes. These mixing times were considered less than optimal based on the laboratory experiments (i.e., 3 hours), but provided a real-world scenario for treating ditches with high discharge rates.

Higher concentrations of chlorpyrifos and diazinon, as well as concentrations of the organophosphates dimethoate, malathion and methyl parathion were detected in Landguard application trials on the lateral ditch (Table 6). Concentrations of chlorpyrifos, diazinon and methyl parathion were significantly reduced by the addition of Landguard. Dimethoate was not reduced with the addition of the enzyme, and concentrations of malathion were reduced in one trial. Cyhalothrin was the only pyrethroid detected during the five lateral ditch trials. It was detected once in a weir sample at a concentration higher than the *H. azteca* immobilization EC50 of 2.3 ng/L (Maund et al., 1998).

All samples collected at the lateral ditch weir were significantly toxic to both test organisms. In the case of *C. dubia*, chlorpyrifos concentrations were high enough to account for most of the observed mortality. Although mixing times were less than one hour, chlorpyrifos concentrations measured at the base of the lateral ditch were less than reporting limits or were not detected. Amphipod toxicity also tracked with chlorpyrifos concentrations. Three out of five of the weir samples contained chlorpyrifos at

concentrations greater than the *H. azteca* LC50 (Table 6). The other two samples were below the LC50, but partial toxic responses were observed.

Table 6. Percent survival (standard deviation) of daphnids and amphipods, and physical and chemical measurements in water samples collected during Landguard application trials in the lateral ditch. ELISA was used to measure chlorpyrifos and diazinon in the first three trials.

6/24/2011	<i>C. dubia</i> (% Survival)		<i>H. azteca</i> (% Survival)		Organophosphates (ng/L)					Pyrethroids (ng/L) CYH	Turbidity (NTU)
	Mean	SD	Mean	SD	CHL	DIA	DIM	MAL	MEP		
B-Weir	0	0	0	0	1558	130	NA	NA	NA	NA	146
C-Bottom	85	10	8	18	<RL	<RL	NA	NA	NA	NA	169
Control	96	9	100	0	ELISA	ELISA					
7/8/2011											
B-Weir	0	0	0	0	388	ND	NA	NA	NA	NA	111
C-Bottom	92	18	4	5	ND	ND	NA	NA	NA	NA	186
Control	100	0	100	0	ELISA	ELISA					
7/12/2011											
B-Weir	0	0	80	12	76	ND	371	Trace	405	ND	194
C-Bottom	76	17	94	9	ND	ND	405	Trace	ND	ND	233
Control	96	9	94	9	ELISA	ELISA					
7/19/2011											
B-Weir	0	0	0	0	99	12	ND	84	ND	8.77	179
C-Bottom	94	9	71	12	ND	ND	ND	81	ND	ND	95
Control	100	0	100	0							
7/21/2011											
B-Weir	0	0	42	22	54	14	ND	82	ND	ND	74
C-Bottom	92	11	66	9	ND	ND	ND	ND	ND	ND	92
Control	92	11	98	4							
<i>C. dubia</i> LC50					53	320		2120		200	
<i>H. azteca</i> E/LC50					86	6510				2.3	

## Overall Treatment Performance

### Vegetated Treatment System

One goal of this study was to optimize the design of the VTS to maximize reduction of total suspended solids and classes of pesticides that have been linked to toxicity in Salinas Valley surface waters (Anderson et al., 2003b; Phillips et al., 2004; Hunt et al., 2008). Improvements to the current system included retention of a higher volume of water in the vegetated portion of the ditch, complete plant coverage throughout the study, and the application of Landguard at the upper end of the vegetated section of the ditch to allow for longer interaction of the enzyme with pesticides.



Modifications of the VTS resulted in minor improvements in water quality relative to the previous trials (Table 7). Because no chlorpyrifos was detected in the VTS, and only low concentrations of diazinon, it was not possible to determine effectiveness of Landguard A900 in the current VTS configuration relative to the previous trials. Results of the laboratory experiments demonstrated that the A900 enzyme was equally effective as the Landguard OP-A enzyme. In addition, the laboratory results suggest that increasing enzyme mixing time by treating runoff as it enters ditch systems will result in greater pesticide hydrolysis. As before, the VTS reduced turbidity, TSS and hydrophobic pesticides under both low and high volume retention schemes. Based on flow, retention time, infiltration rates, percent cover of vegetation in the ditch, and upstream Landguard treatment, the high volume configuration of this integrated VTS likely represents an optimized scenario. The only way to further reduce suspended solids and pyrethroid in run-off would be through application of additional treatments that could include the use of polyacrylimide (PAM) or pyrethroid-specific enzymes.

There was an apparent decrease in the percent removal of the pyrethroid permethrin in the current study. There was 100% removal in two of the three trials that permethrin was measured, but in the third trial there was only 7% reduction (overall average was 77% reduction). Higher concentrations of pyrethroids were detected in the current study. Reductions of organochlorine pesticides and turbidity were not significantly different from the previous study.

Table 7. Average percent reduction by the VTS of various constituents in current study versus previous study (Anderson et al., 2011).

Constituent	Percent Reduction		Highest Concentration (ng/L)	
	Current	Previous	Current	Previous
<b>Organophosphates (with enzyme treatment)</b>				
Chlorpyrifos	NA	98	NA	150
Diazinon	NA	100	NA	2620
<b>Pyrethroids</b>				
Cyhalothrin	99	100	231	64.7
Permethrin	77	100	107	1.18
<b>Organochlorines</b>				
Total DDT	92	96	575	1803
Total Chlordane	100	94	44.3	124
<b>Turbidity</b>	92	88	>1000 NTU	>1000 NTU

Based on the average input rate (0.9 L/s) and the average percent infiltration (42%), hourly mass loadings of the highest chemical concentrations were estimated for the input and output of the VTS. At a peak concentration, cyhalothrin was estimated to enter the VTS at 750 µg/hour, but after passing through the VTS the outflow loading rate was estimated at approximately 7 µg/hour. Similarly, the estimated input loading for total DDT was reduced from 1860 µg/hour to approximately 19 µg/hour in the outflow.

### *Landguard Application in the Lateral Ditch*

Landguard trials in the lateral ditch demonstrated that the enzyme is highly effective at removing diazinon and chlorpyrifos even under high flow, high loading conditions, with minimal mixing times (Table 8). When Landguard was applied to runoff with chlorpyrifos concentrations up to 1558 ng/L and diazinon up to 130 ng/L, pesticides and toxicity were completely removed in the lateral ditch. The application of Landguard effectively eliminated the highest estimated loads of chlorpyrifos and diazinon from 101 mg/hour and 8 mg/hour, respectively. There was also removal of other organophosphates, but these reductions were inconsistent and not related to the type of chemical. For example, malathion was successfully removed in the fourth trial, but not the fifth. A toxic concentration of cyhalothrin was detected at the input in the fourth trial, but was not detected at the bottom of the system. Although there was no vegetation in the lateral ditch system, and the suspended solid concentrations did not go down as the water moved through downstream, this pyrethroid was effectively removed.

Table 8. Average percent reduction of organophosphate and pyrethroids pesticides in the lateral ditch.

Constituent	Percent Reduction	Highest Concentration (ng/L)
<b>Organophosphates (with enzyme treatment)</b>		
Chlorpyrifos	100	1558
Diazinon	100	130
<b>Pyrethroids</b>		
Cyhalothrin	100	8.77
<b>Turbidity</b>	Net Increase	233 NTU

One goal of this study was to optimize the design of an existing integrated VTS. This system was constructed by a cooperating grower to voluntarily reduce sediment and contaminant loads leaving the property. Because of limited space, the original design needed to fit into an existing drainage ditch. The current study improved somewhat on the previous design, but did not reduce all of the measured parameters below the regulatory requirements of the Conditional Waiver of Waste Discharge Requirements for Discharges from Irrigated Lands (Ag Waiver). The Ag Waiver requires that there be no toxicity, but there was significant toxicity in two of the high volume trials (most likely caused by pyrethroids). There are no numerical objectives for pyrethroids, but concentrations of chlorpyrifos and diazinon must be below 25 ng/L and 140 ng/L, respectively. The average detected concentrations of these organophosphates at the bottom of the VTS were 13.7 ng/L for chlorpyrifos and 19.3 ng/L for diazinon. The apparent reduction of these chemicals is related to the low input concentrations, and not the effectiveness of the treatment. In fact, higher concentrations of diazinon were detected in the previous study, and were not effectively treated until the enzyme was used. Much higher concentrations of these chemicals were also detected in the lateral ditch, but were effectively removed by the addition of the enzyme.

The proposed Ag Waiver contains a narrative description for turbidity that states waters shall be free of changes in turbidity that cause nuisance or adversely affect beneficial uses. A narrative objective

indicator of 25 NTU is listed as a target, and this target was not met in several of the VTS trials. The only feasible ways to further reduce particles would be to increase the resident time of the water, reduce water use, or add a flocculating agent such as polyacrylimide (PAM). In the current system, the volume of the vegetated portion of the ditch cannot be increased, but the length of the vegetated portion can be increased by approximately 50 meters. It is unclear whether this increased vegetation would significantly reduce residual turbidity.

### ***Project Outreach***

Four meetings were scheduled to present the results of the current project. The first meeting was conducted at the study site with the cooperating grower, growers from several other operations, and RCD staff. There were twelve attendees that received a two-page handout summarizing the results of the study (Appendix A). After a brief introduction and summary of the previous study, the results of the current study were discussed and questions were answered. A second meeting was conducted at the offices of the California Department of Pesticide Regulation in Sacramento. This meeting was attended by approximately 25 regulators. There were approximately 20 phone attendees who watched the presentation via Web-Ex. The last two meetings were held in conjunction with the University of California Cooperative Extension. The first of these was a presentation to Salinas Valley growers, and was part of the 2012 Irrigation and Nutrient Management Meeting and Cover Crop and Water Quality Field Day. The second meeting took place in San Luis Obispo County. Interested board members and staff from the Central Coast Regional Water Quality Control Board were invited. During these meetings we presented the latest Landguard results, and provided a basis for discussing whether or not Landguard is a viable management practice option for treating pesticides in runoff. Lastly, a special symposium on runoff management practices will be held at the 2012 national meeting of the Society of Environmental Toxicology and Chemistry. The authors will chair this session and present the result of the current study to an international audience.

An important component of all of these meetings was the predicted cost of a typical Landguard application. Currently, there is no published retail price for Landguard A900, but based on previously known prices for Landguard OP-A (2008), and the average discharge recorded in the VTS and lateral ditch, daily costs were calculated. Based on an average daily discharge of 80,000 liters in the VTS and 1.6 million liters in the lateral ditch, and an enzyme dose of 100 µg/L, the average daily estimated cost of treating the VTS would be \$6/day to treat runoff from 120 acres. The average daily cost of treating the lateral ditch, which receives approximately twenty times the runoff, would be \$112/day. CSIRO has also estimated that a single 15 acre crop cycle could be treated for approximately \$100-\$120, but the actual amount would depend on the irrigation practices of the grower.

This study was conducted in California's intensively cultivated Salinas Valley, but project results are transferable to many areas of the country where water quality problems exist downstream of vegetable production. The integrated procedures for using VTS and Landguard will provide technical advisors and

producers with practical, cost-effective tools for treating pesticides of greatest concern in tailwater runoff.

## References

Amweg, E.L., Weston, D.P., 2007. Whole-sediment toxicity identification evaluation tools for pyrethroid insecticides: I. Piperonyl butoxide addition. *Environmental Toxicology and Chemistry* 26, 2389-2396.

Amweg, E.L., Weston, D.P., Ureda, N.M., 2005. Use and toxicity of pyrethroid pesticides in the Central Valley, CA, U.S. *Environmental Toxicology and Chemistry* 24, 966-972.

Anderson, B.S., Hunt, J.W., Phillips, B.M., Nicely, P.A., de Vlaming, V., Connor, V., Richard, N., Tjeerdema, R.S., 2003a. Integrated assessment of the impacts of agricultural drainwater in the Salinas River (California, USA). *Environ. Pollut.* 124, 523-532.

Anderson, B.S., Hunt, J.W., Phillips, B.M., Nicely, P.A., Gilbert, K.D., De Vlaming, V., Connor, V., Richard, N., Tjeerdema, R.S., 2003b. Ecotoxicologic impacts of agricultural drain water in the Salinas River, California, USA. *Environmental Toxicology and Chemistry* 22, 2375-2384.

Anderson, B.S., Phillips, B.M., Hunt, J.W., Connor, V., Richard, N., Tjeerdema, R.S., 2006a. Identifying primary stressors impacting macroinvertebrates in the Salinas River (California, USA): Relative effects of pesticides and suspended particles. *Environ Poll* 141, 402-408.

Anderson, B.S., Phillips, B.M., Hunt, J.W., Largay, B., Shihadeh, R., Berretti, M., 2011. Pesticide and toxicity reduction using an integrated vegetated treatment system. *Environ Toxicol Chem*, 1036-1043.

Anderson, B.S., Phillips, B.M., Hunt, J.W., Worcester, K., Adams, M., Kapellas, N., Tjeerdema, R., 2006b. Evidence of pesticide impacts in the Santa Maria River watershed, California, USA. *Environ Toxicol Chem* 25, 1160-1170.

Ankley, G., Collyard, S., 1995. Influence of Piperonyl Butoxide on the Toxicity of Organophosphate Insecticides to Three Species of Freshwater Benthic Invertebrates. *Comp Biochem Physiol C* 110, 149-155.

Ankley, G.T., Dierkes, J.R., Jensen, D.A., Peterson, G.S., 1991. Piperonyl butoxide as a tool in aquatic toxicological research with organophosphate insecticides. *Ecotoxicol Environ Safety* 21, 266-274.

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

Hunt, J.W., Anderson, B.S., Phillips, B.M., Largay, B., Tjeerdema, R.S., Hanson, E., Berretti, M., Bern, A., 2008. Use of toxicity identification evaluations in determining the pesticide mitigation effectiveness of on-farm vegetated treatment systems. *Environ Poll* 156, 348-358.

Hunt, J.W., Anderson, B.S., Phillips, B.M., Nicely, P.N., Tjeerdema, R.S., Puckett, H.M., Stephenson, M., Worcester, K., De Vlaming, V., 2003. Ambient toxicity due to chlorpyrifos and diazinon in a central California coastal watershed. *Environmental Monitoring and Assessment* 82, 83-112.

- Liess, M., von der Ohe, P.C., 2005. Analyzing effects of pesticides on invertebrate communities in streams. *Environmental Toxicology and Chemistry* 24, 954-965.
- Maund, S.J., Hamer, M.J., Lane, M.C.G., Farrelly, E., Rapley, J.H., Goggin, U.M., Gentle, W.E., 2002. Partitioning, bioavailability, and toxicity of the pyrethroid insecticide cypermethrin in sediments. *Environ Toxicol Chem* 21, 9-15.
- Maund, S.J., Hamer, M.J., Warinton, J.S., Kedwards, T.J., 1998. Aquatic ecotoxicology of the pyrethroid insecticide lambda-cyhalothrin: Considerations for higher-tier aquatic risk assessment. *Pesticide Science* 54, 408-417.
- Moore, M.T., Denton, D.L., Cooper, C.M., Wrynski, J., Miller, J.L., Reece, K., Crane, D., Robins, P., 2008. Mitigation Assessment of Vegetated Drainage Ditches for Collecting Irrigation Runoff. *J Environ Qual* 37, 486-493.
- Pereira, W.E., Domagalski, J.L., Hostettler, F.D., Brown, L.R., Rapp, J.B., 1996. Occurrence and accumulation of pesticides and organic contaminants in river sediment, water and clam tissues from the San Joaquin River and tributaries, California. *Environmental Toxicology and Chemistry* 15, 172-180.
- Phillips, B.M., Anderson, B.S., Hunt, J.W., Nicely, P.A., Kosaka, R.A., Tjeerdema, R.S., de Vlaming, V., Richard, N., 2004. In situ water and sediment toxicity in an agricultural watershed. *Environmental Toxicology and Chemistry* 23, 435-442.
- Phipps, G.L., Mattson, V.R., Ankley, G.T., 1995. The relative sensitivity of three benthic test species to ten chemicals. *Arch Environ Toxicol Chem* 28, 281-286.
- Scholz, N.L., Truelove, N.K., French, B.L., Berejikian, B.A., Quinn, T.P., Casillas, E., Collier, T.K., 2000. Diazinon disrupts antipredator and homing behaviors in chinook salmon (*Oncorhynchus tshawytscha*). *Can. J. Fish. Aquat. Sci.* 57, 1911-1918.
- Sullivan, J.J., Goh, K.S., 2000. Evaluation and validation of a commercial ELISA for diazinon in surface waters. *J Agric Food Chem* 48, 4071-4078.
- SWAMP, 2008. Surface Water Ambient Monitoring Program - Quality Assurance Program Plan Version 1. California Water Boards, Sacramento, CA.
- USEPA, 2000. Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates. EPA/600/R-99/064. Office of Research and Development, Washington D.C.
- USEPA, 2002. Methods for measuring acute toxicity of effluents and receiving water to freshwater and marine organisms. EPA-821-R-02-021. Office of Research and Development, Washington, D.C.
- USEPA, 2010. National Pollutant Discharge Elimination System Test of Significant Toxicity Technical Document. EPA 833-R-10-004. Office of Wastewater Management. Washington DC.

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.

## Appendix A

### *Grower Handout for First Outreach Meeting*

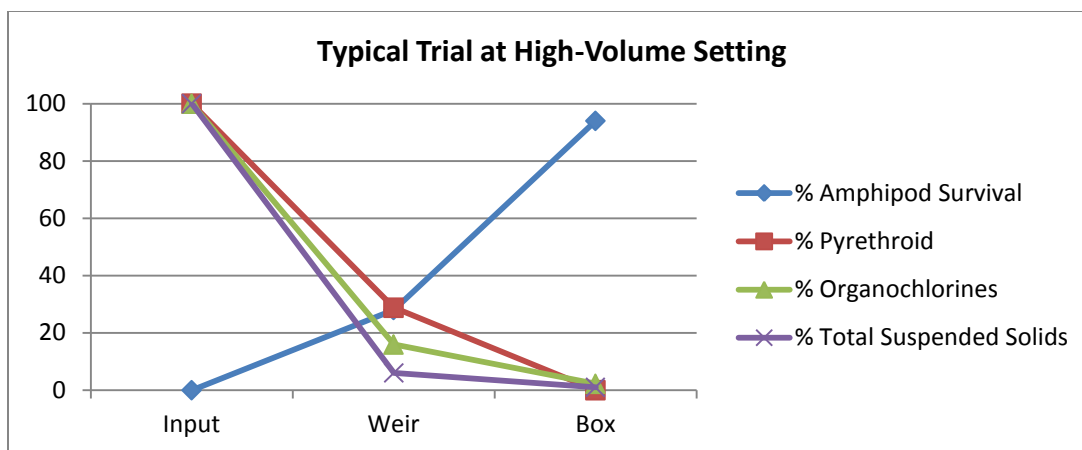
Several experiments were conducted in the Vegetated Treatment System (VTS) and included measurements of toxicity and chemistry at the input A, at the weir B, and at the box C. Two toxicity tests were conducted for each experiment: daphnids (sensitive to organophosphates) and amphipods (sensitive to pyrethroids).



Irrigation runoff enters the VTS at A. Most of the suspended sediment settles out of the water before the weir (B). The water then flows through vegetation (rushes, grass and pennywort) before flowing through the volume control “box” (C).



Two trials were conducted with the VTS at a low-volume setting and four trials were conducted with the VTS at a high volume setting. A greater amount of toxicity and chemistry was reduced at the high-volume setting. The figure below demonstrates the results of a typical trial. Only very low concentrations of organophosphates were detected at the input, but pyrethroids were detected at toxic concentrations in three trials, and were significantly reduced by the VTS (100% reduction). Suspended solids and organochlorine pesticides were also significantly reduced (98-99% reduction).



The VTS was also treated with the Landguard enzyme at the high-volume setting. This enzyme is designed to break down organophosphate pesticides into non-toxic components. Landguard was introduced to the system at the weir at a dose of 100 ug/L of irrigation water. Only very low concentrations of organophosphates were measured in the system during the three Landguard trials. Regardless, pyrethroid pesticides were reduced 98-100%, and all samples collected at the box were non-toxic. ND indicates non-detect. NM indicates not measured.

Trial	Station	Daphnid % Survival	Amphipod % Survival	Cyhalothrin (ng/L)	Permethrin (ng/L)	Turbidity (NTU)
1	B-Weir	0	0	<u>231</u>	2.87	52
	C-Box	84	48	<u>5.03</u>	ND	51
2	B-Weir	84	13	<u>8.2</u>	ND	103
	C-Box	96	91	ND	ND	15
3	B-Weir	92	12	NM	NM	237
	C-Box	100	92	NM	NM	31
Daphnid LC50				200	250	
Amphipod LC50				2.3	21.1	

The same dose of Landguard was also applied to the Lateral Ditch in five separate trials. The discharge in this ditch is up to ten times greater than the VTS, and higher concentrations of organophosphate pesticides were detected in the input water. ND indicates non-detect. NM indicates not measured.

Trial	Station	Daphnid % Survival	Amphipod % Survival	Chlorpyrifos (ng/L)	Diazinon (ng/L)	Malathion (ng/L)	Methyl Parathion (ng/L)	Cyhalothrin (ng/L)
1	B-Weir	0	0	<u>1558</u>	130	NM	NM	NM
	C-Bottom	85	8	<RL	<RL	NM	NM	NM
2	B-Weir	0	0	<u>388</u>	ND	NM	NM	NM
	C-Bottom	92	4	ND	ND	NM	NM	NM
3	B-Weir	0	80	<u>76</u>	ND	Trace	405	ND
	C-Bottom	76	94	ND	ND	Trace	ND	ND
4	B-Weir	0	0	<u>95</u>	ND	84	ND	<u>8.77</u>
	C-Bottom	94	71	ND	ND	81	ND	ND
5	B-Weir	0	42	<u>63</u>	ND	82	ND	ND
	C-Bottom	92	66	ND	ND	ND	ND	ND
Daphnid LC50				53	350	2120		200
Amphipod LC50				86	6510			2.3

### Discharge Rates and Preliminary Cost Estimate

Daily discharge rates were recorded in the VTS and the Lateral Ditch. Based on the average daily rates, and the estimated cost per gram of Landguard (OP-A, 2008 price), the average daily estimated cost of treating the VTS would be \$6/day, whereas the average daily cost of treating the Lateral Ditch would be \$112/day. The Commonwealth Scientific and Industrial Research Organization have also estimated that a single 15 acre crop cycle can be treated for approximately \$100-\$120.

	VTS	Lateral
Average Discharge (Liters per Day)	80,000	1,600,000
Average daily cost to treat discharge based on \$0.70 per gram of Landguard	\$6	\$112