

**Santa Maria River Watershed and Oso Flaco Creek Watershed TMDL  
Monitoring Study – Final Report**

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## **Executive Summary**

Several sites in the Santa Maria River and Oso Flaco Creek watersheds are listed as impaired under section 303(d) of the Clean Water Act and will require development of Total Maximum Daily Load allocations. This project was designed to investigate whether this impairment is due to older legacy pesticides (e.g., organochlorines), as has been shown in historic studies, or whether impairment is due to current use pesticides. Recent studies in these watersheds have shown that water and sediment toxicity is associated with organophosphate and pyrethroid pesticides. The current study investigated temporal and spatial distributions of water and sediment contamination and toxicity at ten sites located throughout the Santa Maria River and Oso Flaco Creek watersheds. The specific project goals were to provide more comprehensive information on the spatial distribution and magnitude of water and sediment toxicity in these watersheds, to identify specific chemicals responsible for toxicity, and to provide additional information on the sources of these chemicals. The latter goal was included to ascertain the relative contributions of pesticides from urban versus agriculture sources. Data from the current project were combined with information from two additional concurrent monitoring programs, a State Water Resources Control Board study of the effects of best management practices on improvements in the Santa Maria River Estuary (BMP Project, Grant Agreement 06-352-553-0), and monitoring conducted by the Central Coast Conditional Waiver Cooperative Monitoring Program (CMP). The ten study sites were divided into four general sub-watersheds to facilitate interpretation of the spatial distributions of pesticide impacts: the lower Santa Maria River Area, the Green Valley Creek Area, the City of Santa Maria Area, and the Oso Flaco Creek Area.

Results from the current study corroborate results of the concurrent BMP and CMP studies in the Santa Maria River and Oso Flaco Creek watersheds. These studies demonstrate that acute toxicity in the Santa Maria River watershed is caused by mixtures of organophosphate and pyrethroid pesticides, and not organochlorine pesticides. The greatest magnitude of contamination and toxicity was observed in the lower Santa Maria River area, where the majority of water and sediment samples were toxic to the invertebrate test species (*Ceriodaphnia dubia* and *Hyalella azteca*). Toxicity identification evaluations (TIEs) showed that water toxicity was caused by diazinon and chlorpyrifos and sediment toxicity was likely caused by chlorpyrifos and pyrethroid pesticide mixtures.

A lower magnitude of toxicity was observed in the Green Valley Creek area. While toxicity in this area was sometimes associated with malathion and chlorpyrifos, toxicity of several of the samples could not be attributed to specific pesticides.

Three stations were located around the City of Santa Maria, and these were intended to represent sites influenced by urban discharges. However, during the course of the study the Blosser Creek and Bradley Channel stations were determined to be influenced by a combination of agriculture and urban runoff. The third station located along the Main Street Ditch was more heavily influenced by urban discharges, but was also likely affected by the adjacent agriculture fields. Data from all three of these stations demonstrated consistent water and sediment toxicity, and toxicity at these stations could be explained by mixtures of the same pesticides observed in the lower Santa Maria River watershed. Water toxicity was likely caused by mixtures of diazinon and chlorpyrifos and sediment toxicity was associated with chlorpyrifos and pyrethroid mixtures. Because diazinon and chlorpyrifos are no longer available to the public for residential use, a likely source of these pesticides is from upstream agricultural runoff which is diverted to these stations. However, two other possible explanations for the presence of organophosphate pesticides in these samples should also be considered. These include inputs associated with commercial pest applicators, and possible use by homeowners having residual stocks of these pesticides. The relative contributions of these possible sources was beyond the scope of the current project, but should be considered as the TMDL process proceeds.

A relatively high magnitude of water toxicity was observed in samples from the Oso Flaco Creek stations and intermittent water toxicity was observed in Oso Flaco Lake. No sediment toxicity was observed in Oso Flaco Lake. The likely causes of toxicity at these stations is unknown because very few of the water samples contained toxic concentrations of pesticides. Data from the CMP showed consistent toxicity at stations above the Oso Flaco Creek station sampled in the current study, but there were no obvious pesticides associated with these samples. Interpretation of data from the current study and the CMP data were limited by the transient nature of the toxicity observed in the laboratory samples, and by the lack of detailed chemical analyses associated with these samples. Given the level of toxicity in the Oso Flaco Creek and Oso Flaco Lake water samples, and the ecological importance of Oso Flaco Lake, causes of toxicity in this

watershed should be emphasized in future studies. This should include more detailed water and sediment chemical analyses and greater emphasis on comprehensive TIEs.

Future studies should also examine the causes of toxicity in the Green Valley Creek area through increased use of chemical analysis and comprehensive TIEs, as well as an investigation into the sources of organophosphate pesticides at stations influenced by urban and agricultural inputs. If management practices are put in place, follow-up monitoring should take place at downstream stations to determine their effectiveness.

One of the primary goals of this project was to assess the relative importance of legacy and current-use pesticides in these watersheds. Previous 303(d) listings have been partly based on detections of organochlorine pesticides in fish tissues. This may continue to be an issue in these watersheds and is currently being investigated in the Santa Maria River Estuary as part of the BMP project. However, none of the organochlorine pesticides were measured at sufficient concentrations to account for the observed toxicity in the current project. The data from this and the other concurrent projects demonstrates that water and sediment toxicity is caused by current-use pesticides (e.g., diazinon, chlorpyrifos, malathion, and pyrethroids). Determining the specific sources of these pesticides should be emphasized as the TMDLs proceed.

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## **Introduction**

Several sites in the Santa Maria River and Oso Flaco Creek watersheds are listed as impaired under section 303(d) of the Clean Water Act and will require development of Total Maximum Daily Load (TMDL) allocations. These include listings for pesticide contamination. The historic data used to support these listings included monitoring conducted as part of the Toxic Substances Monitoring Program (TSM), the California State Mussel Watch Program, and the Bay Protection Toxic Cleanup Program. The 303(d) pesticide listings were based largely on sediment and tissue contaminated by organochlorine pesticides (legacy pesticides). Recent studies conducted by UC Davis for the State Water Resources Control Board, and as part of the monitoring of the Central Coast Conditional Waiver Cooperative Monitoring Program (CMP), have shown that toxicity in these watersheds is largely due to organophosphate pesticides (in water and sediment) and pyrethroid pesticides (in sediment). The current project is designed to (1) provide more comprehensive information on the spatial distribution and magnitude of water and sediment toxicity in watersheds of the Santa Maria River and Oso Flaco Creek, (2) determine temporal variability of water and sediment quality impairments, (3) identify specific chemicals responsible for toxicity, and (4) provide additional information on the sources of these chemicals. This information will be used to support development of pesticide TMDLs in these watersheds.

The study is designed to incorporate data being collected as part of two current monitoring studies in these watersheds: the CMP conducted as part of the Regional Board's Agriculture Waiver agreement, and the Best Management Practices effectiveness monitoring project (BMP) being conducted in the Santa Maria Estuary and its tributaries. This project emphasizes assessments at key inputs affecting the two most ecologically important receiving systems in these watersheds: the Santa Maria River Estuary and Oso Flaco Lake.

The objectives of the study were to develop a Source Analysis for pesticides in the Santa Maria and Oso Flaco watersheds through: 1) determining causes and sources of impairment in the Santa Maria and Oso Flaco watersheds; 2) characterizing geographic distributions, temporal variability and relative magnitudes of toxicity and pesticide contamination from urban and agricultural sources

in the Santa Maria River and Oso Flaco Creek watersheds; 3) collaborating with the Agricultural Program's Cooperative Monitoring Program to partner in data collection and interpretation; and 4) integrating data to develop source analysis for Santa Maria and Oso Flaco TMDLs.

## Methods

### *Study Area*

The Santa Maria River and Oso Flaco Creek watershed study areas are located in northwestern Santa Barbara County and southwestern San Luis Obispo County, California. The watersheds are about 50 miles north of Point Conception and about 150 miles south of Monterey Bay on the central California coast. The current pesticide impairments for these water bodies include DDT, dieldrin, endrin, and chlorpyrifos in the Santa Maria River; DDT, dieldrin, and chlorpyrifos in Orcutt Creek; and dieldrin in Oso Flaco Lake. The greater study area was divided into four sub areas to facilitate the presentation of the monitoring results (Figure 1). The list of stations, sampling dates and parameters assessed on each date are summarized in Table 1.



Figure 1. Google Earth© image of Santa Maria River and Oso Flaco Creek Watershed study sites. Sub areas are delineated in white.

Table 1. List of stations, sample dates, and project tasks.

Station	Station Name	Latitude	Longitude	10/23/08	12/5/08	2/3/09	4/1/09	6/12/09	8/27/09
312BCD	Blosser Channel	34.9792	-120.4529	1	1, 7	1, 2, 3	1, 7	1, 4, 5, 6, 7	1, 7
312BRO	Bradley Channel at River Oaks	34.9742	-120.4245	1	1, 7	1	1, 7	1, 2, 3, 4, 5, 6, 7	1, 7
312MSS	Main Street Ditch	34.9531	-120.4633	1	1, 7	1	1, 7	1, 4, 5, 6, 7	1, 2, 7
312GVS	Green Valley Ck. at Simas	34.9422	-120.5564	1	1, 7	1	1, 7	1, 2, 3, 4, 5, 7	1, 7
312GVT	Orcutt Ck. at Brown Road	34.9340	-120.5579	1	1, 7	1	1, 7	1, 4, 5, 7	1, 2, 7
312OFC	Oso Flaco Ck. at OFC Road	35.0164	-120.5875	1	1, 7	1	1, 7	1, 2, 4, 5, 7	1, 7
312OFL	Oso Flaco Lake	35.0304	-120.6211	1	1, 4, 5	1	1, 4	1, 4, 5, 7	1, 2
312SOL	Solomon Ck. at Sand Plant	34.9573	-120.6317	1	1	1, 2, 3	1	1, 4, 5	1
312ORC	Orcutt Ck. at Sand Plant	34.9575	-120.6323	1	1, 4, 5, 6, 7	1	1, 4, 7	1, 4, 5, 7	1, 2, 7
312SMA	Santa Maria River	34.9611	-120.6414	1	1, 4, 5, 6, 7	1, 2, 3	1, 4, 7	1, 4, 5, 7	1, 7

1 = Water Toxicity, ELISA, and TSS, 2 = Water Chemistry, 3 = Water TIE, 4 = Sediment Toxicity and ELISA, 5 = Sediment Chemistry, Grain Size and TOC, 6 = Sediment TIE, 7 = Discharge

### *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. Bottles 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 the Marine Pollution Studies Laboratory (MPSL). Water samples were stored at  $4 \pm 3^\circ \text{C}$  for no longer than 48 hours prior to toxicity test initiation. Water samples were homogenized and poured through a 25- $\mu\text{m}$  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 was collected to a maximum depth of 5 cm using a polycarbonate core tube. Sediment was placed in 2L glass jars and stored in iced coolers for transport. Testing was initiated within two weeks of sample collection.

### ***Toxicity Testing***

Water toxicity was assessed using 96h exposures with the water flea *Ceriodaphnia dubia* (USEPA, 2002), and sediment toxicity was assessed using 10d exposures with the amphipod *Hyalella azteca* (USEPA, 2000). Ten stations were sampled for water toxicity six times during the 10-month study. Sediment toxicity was assessed at three stations three times and at the remaining seven stations one time. Water quality parameters including dissolved oxygen, pH, conductivity, hardness, alkalinity, and ammonia were measured in both water and sediment tests.

### ***Toxicity Identification Evaluations***

Abbreviated water column TIEs with *C. dubia* were conducted on five toxic samples representing three of the four study areas. TIE exposures were conducted in 20 mL glass scintillation vials (3 replicates) containing 15 mL treated sample and five *C. dubia* neonates. Daphnids were exposed for four days. Several characterization and identification treatments designed to identify organic and pesticide toxicity were performed on a dilution series of the water samples (USEPA, 1991). Sample concentrations were 0 (treatment blank), 25, 50, and 100%. Treatment blanks consisted of control water that underwent the same treatment as the sample. A description of the treatments follows. The Baseline treatment is a test conducted on untreated sample to determine the magnitude of toxicity. Centrifugation was used for samples that appeared to have excess total suspended solids. This treatment reduces toxicity related to particles. Passing the sample through an organic solid-phase extraction (SPE) column removes potentially toxic non-polar organic compounds. 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® water, 25 mL methanol, and 25 mL Nanopure water. After attaching tubing to the columns, they were conditioned by passing two column volumes of acetone, two column volumes of methanol, and one column volume of Nanopure. After conditioning, columns were immediately loaded. A separatory funnel was clamped above the column and filled with 120 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, 120 mL of sample was pumped through. Test concentrations were prepared by combining control and sample rinsates. After extracting the sample, the columns were eluted by first washing with 4 mL Nanopure, followed by 12 mL of acetone. Solvent fractions were evaporated to 1.2 mL and reconstituted in 120 mL clean water. Toxicity of the eluates was tested to assess whether toxic concentrations of organic chemicals were recovered from the columns. Test concentrations were prepared by combining reconstituted fractions with control water containing similar concentrations of solvent.

In addition to the HLB column, an experimental treatment was used to reduce toxicity caused by organic contaminants. Amberlite XAD-4® (Rohm and Haas, Spring House, PA, USA) is a carbonaceous resin used to reduce bioavailability of non-polar organic chemicals (Kosian *et al.*, 1999). Approximately 4g of resin was activated by rinsing with 4 mL of methanol and then thoroughly rinsed with Nanopure water. The resin was then added to 120 mL of sample, stirred, and allowed to equilibrate for 24 hours. The resin was then separated from the sample and the sample was diluted for testing. The resin was eluted by placing it in a syringe and passing 10 mL of acetone through the resin at a rate of 1 mL/min. The acetone was evaporated to 1.2 mL and added to 120 mL of clean water for dilution and testing.

Samples also underwent recently developed treatments for the characterization and identification of pyrethroid pesticide toxicity. The addition of carboxylesterase enzyme (Sigma-Aldrich, St. Louis, MO, USA) to the sample hydrolyzes ester-containing compounds, such as pyrethroid pesticides to their corresponding acid and alcohol, which are generally not toxic (Wheelock *et al.*, 2004). A bovine serum albumin (BSA) protein-addition control was conducted with this treatment to account for reduction of contaminant bioavailability due to complexation by the

enzyme addition. Piperonyl butoxide (PBO, Sigma-Aldrich, St. Louis, MO, USA) is a metabolic inhibitor used to block the metabolic activation of acetylcholinesterase-inhibiting organophosphate pesticides (Ankley et al., 1991b). It is also a potent synergist of pyrethroid toxicity, because it inhibits their metabolism (Kakko *et al.*, 2000; Ware and Whitacre, 2004). PBO (250 µg/L) was added to water samples to reduce toxicity caused by organophosphate pesticides and increase toxicity caused by pyrethroids.

The whole sediment TIEs consisted of five replicate 250 mL beakers containing 50 mL sediment and approximately 200 mL overlying water and ten amphipods. Sediment TIE treatments included 10% Amberlite addition to reduce the bioavailability of organic contaminants (USEPA, 2007). Carboxylesterase enzyme, BSA and PBO were all added to overlying water in separate treatments to assess the potential contribution of pyrethroid and organophosphate pesticides to the observed toxicity.

### ***Chemical and Physical Analysis***

All water samples and interstitial water from sediment samples were analyzed for total concentrations of the organophosphate pesticides diazinon and chlorpyrifos 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 precision was determined with duplicate measurements. 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. The ELISA concentrations were used to calculate toxic units for each pesticide (1 TU = measured concentration divided by either the diazinon or chlorpyrifos LC50). Greater than 50% mortality would be expected in samples with greater than one TU of organophosphate pesticide. When both chlorpyrifos and diazinon were detected in a given water sample, the TUs for each were added to provide a combined TU value, because previous research has demonstrated that the toxicity of diazinon and chlorpyrifos is additive when they co-occur (Bailey et al., 1997).

In addition to the ELISA analysis for chlorpyrifos and diazinon, one water sample from each site was also analyzed for organophosphate pesticides (EPA Method 625M) and pyrethroid pesticides (EPA Method 625M Negative Chemical Ionization (NCI)). One sediment sample from each site was analyzed for organophosphate pesticides, organochlorine pesticides, arochlor PCBs, and PCB congeners (EPA Method 8270CM), and pyrethroid pesticides (EPA Method 8270CM NCI). All analyte identifications were confirmed by Gas Chromatography/Mass Spectroscopy (GC/MS). A complete list of analytes is included in Table A1.

Water samples were analyzed for total suspended solids (TSS, Method SM 2540D), and sediment samples were analyzed for total organic carbon (TOC, EPA Method 9060A) and grain size (Method SM 2540D).

### ***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). The acceptability of each TIE treatment was evaluated by checking for adequate survival in each treatment blank. Water treatments were evaluated using toxic units based on the results of the dilution series. These units were calculated by dividing 100 by the treatment-specific median lethal concentration (LC50, as percent sample) calculated from the sample dilution series. Comparing toxic units among the treatments provided better resolution than simply comparing single concentrations from the various dilution series.

All concentrations of pesticides measured in water and sediment were compared to published toxicity thresholds and regulatory criteria provided by the Regional Board staff. The thresholds used for comparison to the water chemistry data are provided in Table 2, and include individual LC50 data from published studies and various regional water quality criteria. Because chemical analyses were conducted on grab samples, CCC and CMC criteria should only be used qualitatively. The Central Valley Regional Water Quality Control Board's (CVRWQCB) draft pesticide water quality criteria for the protection of aquatic life are also listed for reference

(<http://www.waterboards.ca.gov/>). Sediment toxicity thresholds and sediment quality guideline values used to assess the sediment chemistry data are provided in Table 3.

Table 2. Water chemistry evaluation thresholds and criteria. LC50 indicates median lethal concentration for *C. dubia*. CMC indicates criterion maximum concentration (1- hour average). Not to be exceeded more than once in a three year period. CCC indicates criterion continuous concentration (4-day (96-hour) average). Not to be exceeded more than once in a three year period. CVRWQCB = Central Valley Regional Water Quality Control Board (Region 5). These draft criteria are included for comparison to the LC50 and other values considered in this report.

Chemical	ng/L	Endpoint	Reference	CVRWQCB Draft Pesticide Water Quality Criteria (ng/L)			
<b>Organophosphates</b>							
Chlorpyrifos	53	LC50	(Bailey et al., 1997)	10			
	83	CMC	Squirt Water Quality Criteria				
	41	CCC	Squirt Water Quality Criteria				
Diazinon	25	CMC	Santa Maria Preliminary Numeric Target (acute)	200			
			Region 3, 303d listing criterion				
			Santa Maria Preliminary Numeric Target (chronic)				
	15	CCC	(Bailey et al., 1997)				
	320	LC50	Squirt Water Quality Criteria				
	100	CMC	Squirt Water Quality Criteria				
Dichlorvos	100	CCC	Santa Maria Preliminary Numeric Target (acute)	150			
			Region 3, 303d listing criterion				
			Santa Maria Preliminary Numeric Target (chronic)				
Malathion	130	LC50	(Ankley et al., 1991a)	150			
	2120	LC50	(Ankley et al., 1991a)				
<b>Pyrethroids</b>	100	CCC	Squirt Water Quality Criteria	4			
			Bifenthrin		142	LC50	(Wheelock et al., 2004)
			Cyfluthrin		344	LC50	(Wheelock et al., 2004)
			Cypermethrin		683	LC50	(Wheelock et al., 2004)
			Lambda Cyhalothrin		200	LC50	(Wheelock et al., 2004)
Permethrin	250	LC50	(Wheelock et al., 2004)	1			

Table 3. Sediment chemistry evaluation thresholds. LC50 indicates median lethal concentration. TEL indicates threshold effects level. PEL indicates probable effects level. PEC indicates probable effects concentration.

Chemical	ng/g	µg/g oc	Endpoint	Reference
<b>Organophosphates</b>				
Chlorpyrifos	399	1.77	LC50	(Brown et al., 1997; Amweg and Weston, 2007)
<b>Pyrethroids</b>				
Bifenthrin	12.9	0.52	LC50	(Amweg et al., 2005)
Cyfluthrin	13.7	1.08	LC50	(Amweg et al., 2005)
Cypermethrin	14.87	0.38	LC50	(Maund et al., 2002) mean value
Esfenvalerate	41.8	1.54	LC50	(Amweg et al., 2005)
Lambda-Cyhalothrin	5.6	0.45	LC50	(Amweg et al., 2005)
Permethrin	200.7	10.83	LC50	(Amweg et al., 2005)
<b>Organochlorines</b>				
Dieldrin		2000	Mean LC50	(USEPA, 2003)
	2.85		TEL	Squirt Water Quality Criteria
	6.67		PEL	Squirt Water Quality Criteria
Total Chlordane	17.6		PEC	(Macdonald, 2000)
	4.5		TEL	Squirt Water Quality Criteria
	8.9		PEL	Squirt Water Quality Criteria
Total DDT	572		PEC	(Macdonald, 2000)
	11000	367	LC50	(Nebeker et al., 1989) 3% TOC
	49700	473	LC50	(Nebeker et al., 1989) 10.5% TOC
		2580	LC50	(Swartz et al., 1994)
	6.98		TEL	Squirt Water Quality Criteria
DDD	4450		PEL	Squirt Water Quality Criteria
		1300	LC50	Predicted in Weston et al. 1994 (Amweg et al., 2005)
4,4'-DDD	3.54		TEL	Squirt Water Quality Criteria
	8.51		PEL	Squirt Water Quality Criteria
DDE		8300	LC50	Predicted in Weston et al. 1994
	1.42		TEL	Squirt Water Quality Criteria
4,4'-DDE			PEL	Squirt Water Quality Criteria
	6.75		PEL	Squirt Water Quality Criteria

### ***Discharge Measurement***

Instantaneous discharge was generally calculated by taking ten cross sectional flow measurements at each station on each date where discharge was characterized. In some cases where flow was difficult to measure, discharge was estimated by measuring cross-sectional flow in a culvert. Loading for diazinon and chlorpyrifos was calculated by multiplying discharge by the ELISA concentration. Where data were available, similar calculations were made for pesticides measured using GC/MS.

## 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. Concurrent 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 2), indicating that test organisms responded to the toxicant in a manner consistent with previous tests.

Three field duplicates were tested for water toxicity and one field duplicate was tested for sediment toxicity. One water toxicity duplicate from the 312SMA station had a relative percent difference of 50%, which most likely indicates the heterogeneity of the signal in the river. The relative percent difference between the other two water toxicity duplicates was 4% or less. The difference between the two sediment duplicates was 0% because both samples caused complete mortality.

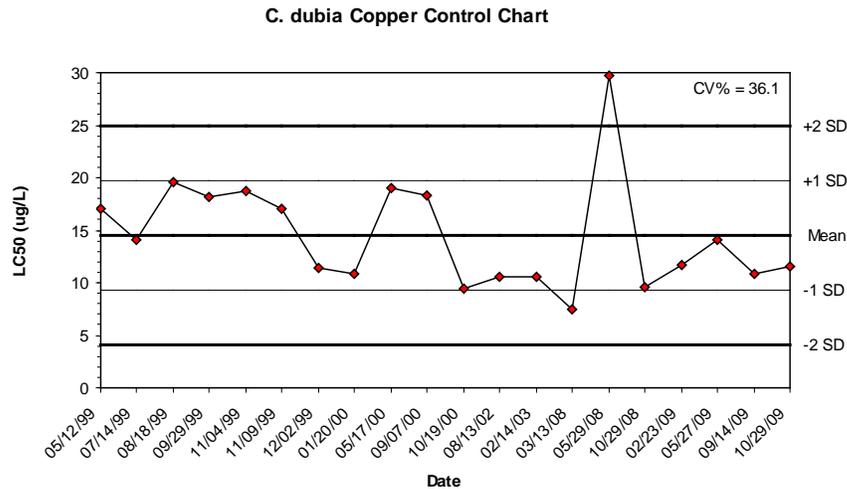


Figure 2a. *C. dubia* reference toxicant control chart.

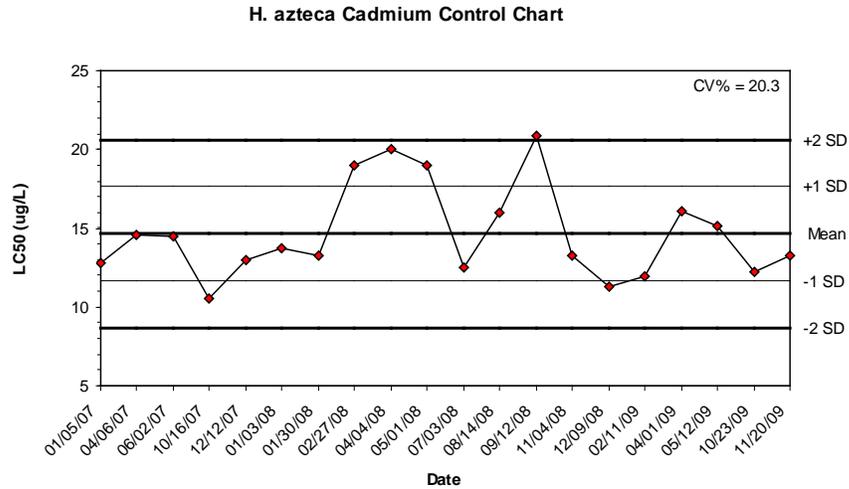


Figure 2b. *H. azteca* reference toxicant control chart.

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 93% to 140% for chlorpyrifos and 92% to 120% for diazinon, and relative percent differences between ELISA duplicates were 19% or less. Ten ELISA measurements were compared to corresponding measurements with GC/MS (Table 4). Five chlorpyrifos and seven diazinon ELISA measurements were non-detect (<50 and <30 ng/L, respectively), and were in agreement with corresponding GC/MS measurements. Five detected ELISA chlorpyrifos measurements ranged from -46% to 44% of the GC/MS measurements. Three detected ELISA diazinon measurements ranged from 67% to 251% of the GC/MS measurements. The ELISA chlorpyrifos concentrations generally agreed with the GC/MS concentrations, but the ELISA diazinon concentrations tended to overestimate the GC/MS concentrations. It should be noted that the reporting limit for chlorpyrifos is greater than the test organism water toxicity thresholds, and concentrations listed as less than the reporting limit could still be contributing to toxicity.

Table 4. Comparison of GC/MS and ELISA measurements of chlorpyrifos and diazinon.

Station	Date	Chlorpyrifos (ng/L)			Diazinon (ng/L)		
		GC/MS	ELISA	RPD	GC/MS	ELISA	RPD
312BCD	03/Feb/2009	114.6	141	23	5.6	<30	
312BRO	10/Jun/2009	69.8	92 (<RL)	32	66.3	111	67
312GVS	10/Jun/2009	31	<50		22.3	<30	
312GVT	26/Aug/2009	19.4	<50		10.6	<30	
312MSS	26/Aug/2009	ND	<50		ND	<30	
312OFC	10/Jun/2009	11.8	<50		ND	<30	
312OFL	26/Aug/2009	ND	<50		ND	<30	
312ORC	26/Aug/2009	219.8	317	44	ND	<30	
312SMA	03/Feb/2009	1133	897	-21	20.7	48 (<RL)	132
312SOL	03/Feb/2009	1874.4	1013	-46	20.8	73	251

GC/MS chemistry precision and accuracy were evaluated through the analysis of external laboratory reference materials, blank spikes, matrix spikes, and the measurement of sample duplicates. GC/MS reference material recoveries in deionized water were all within acceptable ranges except for azinphos methyl in the water analysis and dicofol in the sediment analysis (see chemistry database). Azinphos methyl was not detected in water samples and detected dicofol concentrations might be overestimated in sediment samples. In the matrix spikes using sediment from 312MSS and 312ORC, there were matrix interferences with several organochlorine and organophosphate chemicals. Several spiked concentrations of organochlorines were below the acceptable range in both sediments, and several spiked concentrations of pyrethroids were above the acceptable range in 312ORC. Relative percent differences between GC/MS duplicates were 30% or less in water samples, but because of the matrix spike interferences in the sediment, some organochlorine duplicates exceeded the acceptable limit of 30%. According to the analytical laboratory, the surrogate compound recovery was out of control due to matrix interference, but the associated method blank surrogate compound was in control and therefore the sample data was reported without further clarification. The reported concentrations for organochlorine compounds in sediment should be acceptable for analysis.

The relative percent differences between grain size duplicates were all less than 8%, and the relative percent differences between TOC and TSS duplicates were both less than 11%.

### *Physical Characteristics of Water and Sediment*

Total suspended sediment concentrations ranged from 1.3 to 1353 mg/L (Table 5). The highest concentrations of suspended sediments were observed at 312OFC. Suspended sediments did not correlate with observed toxicity or detected chemicals in water samples.

Table 5. Total Suspended Solid (TSS) concentrations (mg/L).

Station	10/23/2008	12/5/2008	2/3/2009	4/1/2009	6/12/2009	8/27/2009
312SOL	597	17.3	418	135	90.0	104
312ORC	13.8	12.0	302	116	44.0	87.0
312SMA	44.5	44.3	636	68.0	13.6	51.0
312GVS	2.0	1.3	2.3	4.0	96.5	10.3
312GVT	4.3	12.3	58.5	536	39.2	43.3
312OFC	1353	663	18.5	359	47.5	969
312OFL	15.0	9.7	19.3	18.0	25.3	29.5
312BRO	3.8	53.7	12.3	29.8	24.0	9.2
312BCD	10.6	12.0	5.7	18.5	285	13.8
312MSS	30.3	5.0	23.8	56.3	75.6	6.0

Total organic carbon and grain size data are summarized in Table 6. Carbon concentrations ranged from 0.69% to 7.71% and were used to normalize total sediment chemical concentrations to organic carbon-corrected concentrations using the following equation:

$$\text{Chemical Concentration } (\mu\text{g/g dry wt.}) \div \text{TOC } (\%) = \text{OC-Corrected Concentration } (\mu\text{g/g OC})$$

Corrected concentrations are considered to be more representative of the bioavailable fraction of contaminants in sediment. Higher concentrations of TOC can reduce the bioavailability of sediment contaminants. Most of the sediment samples had concentrations of fine grains greater than 50%, the target concentration for fine-grained sediments recommended by the Surface Water Ambient Monitoring Program (SWAMP). Sediment from 312OFC and 312OFL had concentrations of sand ranging from approximately 55% to 69%, and one of the samples from 312SMA, normally an area of fine-grain deposition, contained over 70% sand, but also a higher concentration of TOC than the other 312SMA sample. Fine-grained sediment from 312SMA generally contained higher concentrations of contaminants than the coarse-grained sample.

Table 6. Total organic carbon concentrations (%) and particle size fractions (%) from sediment samples. Percent fines are the sum of percent silt and percent clay.

Station	TOC (%)	Sand (%)	Silt (%)	Clay (%)	% Fines
312SOL	1.03	36.25	48.06	15.68	63.75
312ORC	0.78	34.21	52.37	13.42	65.79
312SMA 1	2.03	7.27	74.22	18.51	92.73
312SMA 2	2.99	70.31	29.1	0.59	29.69
312GVS	1.50	22.03	64.14	13.83	77.97
312GVT	1.52	30.26	56.69	13.05	69.74
312OFC	0.69	54.94	34.31	10.75	45.06
312OFL 1	1.73	56.80	37.77	5.42	43.20
312OFL 2	3.67	68.7	30.6	0.7	31.3
312BRO	3.31	39.47	53.47	7.05	60.53
312BCD	7.71	49.64	47.16	3.20	50.36
312MSS	3.43	24.46	65.16	10.39	75.54

### ***Lower Santa Maria River Area***

#### *Area Description*

Water from Solomon Creek (312SOL) joins with Orcutt Creek approximately 120 m upstream of the Orcutt Creek station (312ORC). Water at 312SOL was turbid, compared to the relatively clear water flowing in Orcutt Creek. Average total suspended solids concentrations in Solomon Creek were approximately 2.5 times the TSS in Orcutt Creek (Table 7). Station 312ORC is approximately 0.5 km from the confluence of Orcutt Creek and the Santa Maria River. The Santa Maria station sampled in this study (312SMA) is approximately one km downstream of the confluence of Orcutt Creek and the river. Approximately 90% of the dry-weather flow observed in the Maria River at 312SMA is comprised of discharge from the drainage ditch that enters the river near the Guadalupe Dunes Reserve kiosk, combined with the flows of Solomon and Orcutt Creeks (SAIC, 2004).

#### *Water Toxicity and Chemistry*

Samples from 312SOL and 312SMA were toxic on every sampling event (Figure 3, Table 7), while samples from 312ORC were toxic on three of the five sampling events. Concentrations of chlorpyrifos and diazinon, as measured by ELISA, were high enough in 12 of the 15 toxic samples to account for the observed toxicity to *C. dubia*. The toxicity of the three remaining samples was probably caused by the additive toxicity of these organophosphate pesticides, but the ELISA concentrations were below the reporting limits, and therefore estimated.

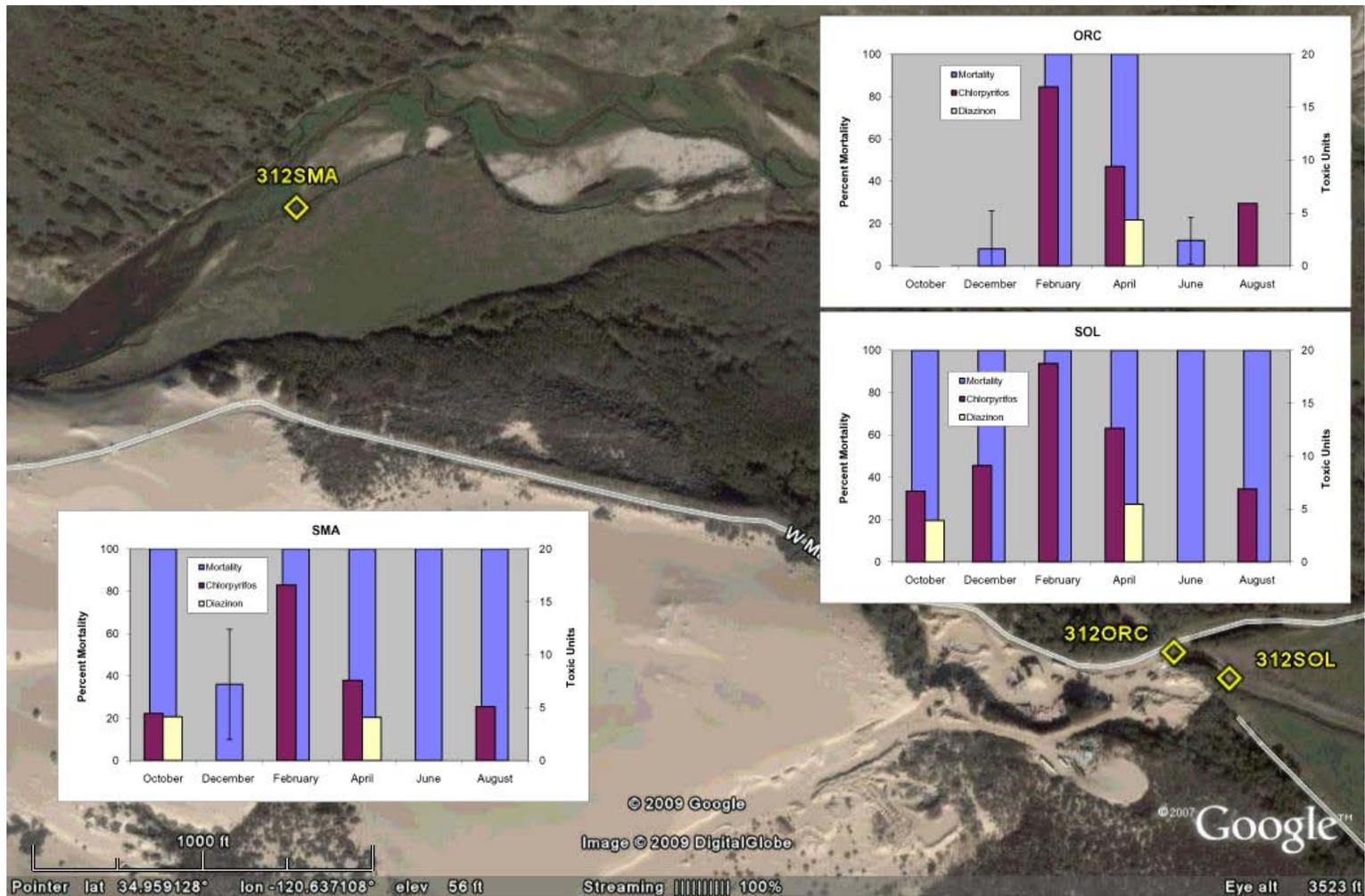


Figure 3. Google Earth© image of the lower Santa Maria River area. Inset figures summarize water toxicity results (error bars indicate standard deviation) and ELISA measurements of chlorpyrifos and diazinon concentrations expressed in toxic units.

Three selected water samples from this area were analyzed for a suite of organophosphate and pyrethroid pesticides using GC/MS. Stations 312SOL and 312SMA were analyzed during the third sampling event and station 312ORC was analyzed during the sixth sampling event. Several chemicals were detected in these samples, but only concentrations of chlorpyrifos were greater than the toxicity thresholds for *C. dubia* (Table 7). According to the GC/MS results, water from stations 312SOL, 312ORC and 312SMA contained approximately 35, 4 and 21 TUs of chlorpyrifos, respectively. Water from 312ORC also contained approximately 0.7 TUs of malathion. Low ng/L concentrations of pyrethroid pesticides were also detected in all three water samples. Several of these concentrations were below reporting limits, and therefore estimated. Although these concentrations were below the toxicity threshold for *C. dubia*, they are in the range of concentrations that would be toxic to *H. azteca* in water exposures. This is especially true if the additive toxicity of pyrethroids is considered.

Table 7. Lower Santa Maria Area. Mean *C. dubia* survival (standard deviation), concentrations of chlorpyrifos and diazinon as measured by ELISA, and detected chemicals in select water samples as measured by GC/MS. All concentrations are in ng/L. Shading indicates significant toxicity or chemical concentrations greater than the *C. dubia* LC50. ND indicates non-detectable concentration. Bold values are below reporting limit, but above detection limit and are therefore estimated.

Date	312SOL			312ORC			312SMA		
	Survival (SD)	Chlor.	Diaz.	Survival (SD)	Chlor.	Diaz.	Survival (SD)	Chlor.	Diaz.
10/23/2008	0 (0)	361	1310				0 (0)	241	1384
12/5/2008	0 (0)	491	ND	92 (18)	<b>51</b>	ND	64 (26)	<b>67</b>	ND
2/3/2009	0 (0)	1013	73	0 (0)	921	<b>43</b>	0 (0)	897	<b>48</b>
4/1/2009	0 (0)	692	1832	0 (0)	508	1458	0 (0)	410	1368
6/12/2009	0 (0)	<b>55</b>	<b>41</b>	88 (11)	ND	ND	0 (0)	<b>88</b>	<b>33</b>
8/27/2009	0 (0)	371	ND	0 (0)	317	ND	0 (0)	276	ND
<b>Detected Chemicals</b>	2/3/2009			8/27/2009			2/3/2009		
<b>Organophosphates</b>	ng/L			ng/L			ng/L		
Chlorpyrifos	1874			219.8			1133		
Diazinon	20.8			ND			20.7		
Dimethoate	ND			8			ND		
Malathion	ND			1497			19.6		
<b>Pyrethroids</b>									
Cyfluthrin	ND			2.1			ND		
Cypermethrin	4.2			ND			3.5		
Esfenvalerate	<b>1.9</b>			ND			<b>1.2</b>		
Fenvalerate	<b>0.9</b>			ND			<b>0.8</b>		
Fluvalinate	ND			ND			<b>0.5</b>		
L-Cyhalothrin	<b>1.9</b>			<b>0.7</b>			2.1		
Resmethrin	ND			<b>14.3</b>			ND		

*Water Toxicity Identification Evaluations*

Toxicity identification evaluations were conducted on water from 312SOL and 312SMA in February 2009. The results of both TIEs were similar. The samples were very toxic ( $\geq 8$  TUs) and contained concentrations of chlorpyrifos well above the *C. dubia* LC50 (Table 8).

Removing suspended sediments with centrifugation reduced the chlorpyrifos concentrations, but not below toxic levels. Passing the samples through the HLB columns completely removed toxicity and chlorpyrifos. The column eluates were both significantly toxic and contained toxic concentrations of chlorpyrifos. Addition of Amberlite to the 312SMA sample removed approximately two-thirds of the chlorpyrifos, but did not reduce toxicity. The Amberlite eluate was significantly toxic. Addition of enzyme somewhat reduced toxicity in the 312SMA sample, but based on concentrations of pyrethroids, it is likely this reduction was due to the binding of chlorpyrifos with the enzyme. Addition of PBO should reduce toxicity caused by organophosphate pesticides, but it is likely the concentration of PBO added to the samples was overwhelmed by the concentrations of chlorpyrifos and other organophosphates. Bailey et al. (Bailey et al., 1996) reported 40% *C. dubia* survival in 750 ng/L chlorpyrifos when 200  $\mu\text{g/L}$  PBO was added. The current TIEs used 250  $\mu\text{g/L}$  PBO, but the chlorpyrifos concentrations were 1133 ng/L and 1874 ng/L for 312SOL and 312SMA, respectively (Table 8). TIE treatments and chemical analysis indicate that chlorpyrifos was the primary cause of toxicity in these samples.

Table 8. Lower Santa Maria Area. Mean percent (standard deviation) survival of *C. dubia* in water toxicity identification evaluation (TIE) treatments. Toxic units are calculated from the dilution series by dividing the LC50 (in percent) by 100. ELISA chlorpyrifos concentrations and other detected chemicals are listed with chemical LC50s for comparison. Shading indicates concentrations that have exceeded the *C. dubia* LC50. NA indicates not analyzed. ND indicates not detected.

312SOL (2/6/09) Treatment	Mean Percent (SD) Survival				Toxic Units	ELISA Chlor. ng/L	Other Detected Chemicals	Concentration ng/L	LC50 ng/L
	Control	25%	50%	100%					
Baseline	87 (12)	0 (0)	0 (0)	0 (0)	$\geq 8$	2469	Chlorpyrifos	1874	53
Centrifuge	93 (12)	0 (0)	0 (0)	0 (0)	$\geq 8$	1725	Diazinon	20.8	320
HLB Column	93 (12)	93 (12)	93 (12)	93 (12)	$< 1$	ND	Cypermethrin	4.2	683
HLB Eluate	93 (12)	0 (0)	0 (0)	0 (0)	$\geq 8$	1005	Esfenvalerate	<b>1.9</b>	
Enzyme	100 (0)	0 (0)	0 (0)	0 (0)	$\geq 8$	2274	Fenvalerate	<b>0.9</b>	
BSA	100 (0)	0 (0)	0 (0)	0 (0)	$\geq 8$	2364	L-Cyhalothrin	<b>1.9</b>	200
PBO	93 (12)	0 (0)	0 (0)	0 (0)	$\geq 8$	2343			

312SMA (2/10/09) Treatment	Mean Percent (SD) Survival				Toxic Units	ELISA Chlor. ng/L	Other Detected Chemicals	Concentration ng/L	LC50 ng/L
	Control	25%	50%	100%					
Baseline	93 (12)	0 (0)	0 (0)	0 (0)	≥8	1212	Chlorpyrifos	1133	53
Centrifuge	100 (0)	0 (0)	0 (0)	0 (0)	≥8	928	Diazinon	20.7	320
HLB Column	100 (0)	93 (12)	93 (12)	93 (12)	<1	ND	Malathion	19.6	2120
HLB Eluate	100 (0)	0 (0)	0 (0)	0 (0)	≥8	912	Cypermethrin	3.5	683
Amberlite	93 (12)	0 (0)	0 (0)	0 (0)	≥8	464	Esfenvalerate	<b>1.2</b>	
Amb. Eluate	100 (0)	NA	NA	0 (0)	NA	NA	Fenvalerate	<b>0.8</b>	
Enzyme	100 (0)	80 (35)	0 (0)	0 (0)	3.1	1226	Fluvalinate	<b>0.5</b>	
BSA	93 (12)	0 (0)	0 (0)	0 (0)	≥8	1262	L-Cyhalothrin	2.1	200
PBO	93 (12)	0 (0)	0 (0)	0 (0)	≥8	1172			

### *Sediment Toxicity and Chemistry*

Sediment from 312SOL was sampled once for toxicity testing and chemical analysis, and sediment from 312ORC and 312SMA was sampled three times. Two sediment samples from 312SMA and one sample from the other two stations were analyzed for pesticides. All seven samples were significantly toxic and had interstitial water concentrations of chlorpyrifos greater than the toxicity threshold for *H. azteca* (Table 9). In addition to chlorpyrifos, several pyrethroid and organochlorine pesticides were also detected. None of the total concentrations exceeded whole sediment toxicity thresholds for *H. azteca*, but when the concentrations were corrected for organic carbon content, the concentration of chlorpyrifos exceeded the organic carbon-corrected LC50 in all three samples. Two organic carbon-corrected concentrations of pyrethroids also exceeded LC50s: cypermethrin at 312ORC and lambda-cyhalothrin at 312SMA.

Table 9. Lower Santa Maria Area. Mean *H. azteca* survival (standard deviation), interstitial water concentrations of chlorpyrifos and diazinon as measured by ELISA (ng/L), and detected chemicals in select samples as measured by GC/MS. Detected chemical concentrations are presented in total sediment concentrations (ng/g) and concentrations corrected for organic carbon content ( $\mu\text{g/g oc}$ ). Shading indicates significant toxicity or chemical concentrations greater than the *H. azteca* LC50. ND indicates non-detectable concentration. Bold values are below reporting limit, but above detection limit and are therefore estimated.

Date	312SOL			312ORC			312SMA			
	Survival (SD)	Chlor.	Diaz.	Survival (SD)	Chlor.	Diaz.	Survival (SD)	Chlor.	Diaz.	
12/9/2008				3 (7)	134	ND	0 (0)	721	<b>53</b>	
4/3/2009				8 (14)	325	946	0 (0)	589	491	
6/19/2009	5 (5)	173	<b>41</b>	3 (5)	387	<b>52</b>	5 (8)	108	<b>39</b>	
<b>Detected Chemicals</b>	6/19/2009			12/9/2008			12/9/2008		6/19/2009	
<b>Organophosphates</b>	ng/g	$\mu\text{g/g oc}$		ng/g	$\mu\text{g/g oc}$		ng/g	$\mu\text{g/g oc}$	ng/g	$\mu\text{g/g oc}$
Chlorpyrifos	41.4	4.02		38.5	4.94		164.4	8.10	58.9	1.97
<b>Pyrethroids</b>										
Cypermethrin	2.3	0.22		3.5	0.45		6.2	0.31	11	0.37
Esfenvalerate	1.6	0.16		2.8	0.36		5.5	0.27	2.8	0.09
Fenvalerate	0.9			1.2			2.4		2.4	
L-Cyhalothrin	1.4	0.14		2.9	0.37		14.1	0.69	2.4	0.08
<b>Organochlorines</b>										
2,4'-DDD	18			10.3			29.5		20	
2,4'-DDE	4.4			2.5			6.5		6.1	
2,4'-DDT	ND			ND			3		ND	
4,4'-DDD	32.6			21			68.9		31.6	
4,4'-DDE	207.5			130.1			337.6		275.3	
4,4'-DDT	5.8			3.2			10.1		7.6	
Total DDT	268.3	26.05		167.1	21.42		455.6	22.44	340.6	11.39
Dicofol	48.8			ND			10.3		61.4	
Toxaphene	ND			88.63			551.92		ND	

#### *Sediment Toxicity Identification Evaluations*

Stations 312ORC and 312SMA were selected for whole sediment TIEs designed to characterize the cause of toxicity. The addition of Amberlite resin to 312ORC sediment significantly increased survival, indicating toxicity was caused by an organic contaminant (Table 10). Amberlite addition also increased survival in 312SMA sediment, but the response was not significantly different from the baseline. Addition of enzyme slightly increased survival in 312ORC sediment, but the response was not significantly different from the baseline sample, nor was it significantly different from the response in the BSA treatment. Addition of PBO did not reduce toxicity. It is likely that neither of these treatments provided conclusive evidence because

there were toxic concentrations of both chlorpyrifos and a pyrethroid in these samples. Toxicity in these samples was likely caused by a combination of chlorpyrifos and pyrethroids (Table 10).

Table 10. Lower Santa Maria Area. Mean percent (standard deviation) survival of *H. azteca* in sediment toxicity identification evaluation (TIE) treatments, and detected organic carbon-corrected chemical concentrations that exceeded *H. azteca* LC50 values (indicated by shading). \* indicates significant reduction of toxicity and significantly different from the dilution control. \*\* indicates significant reduction of toxicity and significantly different from BSA.

Treatment	312ORC Mean (SD)	312SMA Mean (SD)
Sample Baseline	0 (0)	0 (0)
10% Amberlite	86 (13)*	22 (28)
Control (10% Amberlite)	100 (0)	100 (0)
Enzyme	10 (14)	0 (0)
Control (Enzyme)	94 (9)	94 (9)
Bovine Serum Albumin (BSA)	6 (9)	0 (0)
Control (BSA)	98 (4)	98 (4)
Piperonyl Butoxide (PBO)	0 (0)	0 (0)
Control (PBO)	96 (5)	96 (5)
Dilution Control	0 (0)	0 (0)
Control	100 (0)	100 (0)
Chemical	Organic Carbon-Corrected Concentrations (µg/g OC)	
Chlorpyrifos	4.94	8.10
Cypermethrin	0.45	0.31
L-Cyhalothrin	0.37	0.69

### ***Green Valley Creek Area***

#### *Area Description*

Green Valley Creek is a tributary to Orcutt Creek, and the confluence of these creeks is approximately 7.3 km upstream of station 312ORC. Green Valley Creek at Simas Road (312GVS) and Orcutt Creek at Brown Road (312GVT) are approximately 0.8 km and 0.95 km upstream of the confluence of the creeks, respectively (Figure 4).

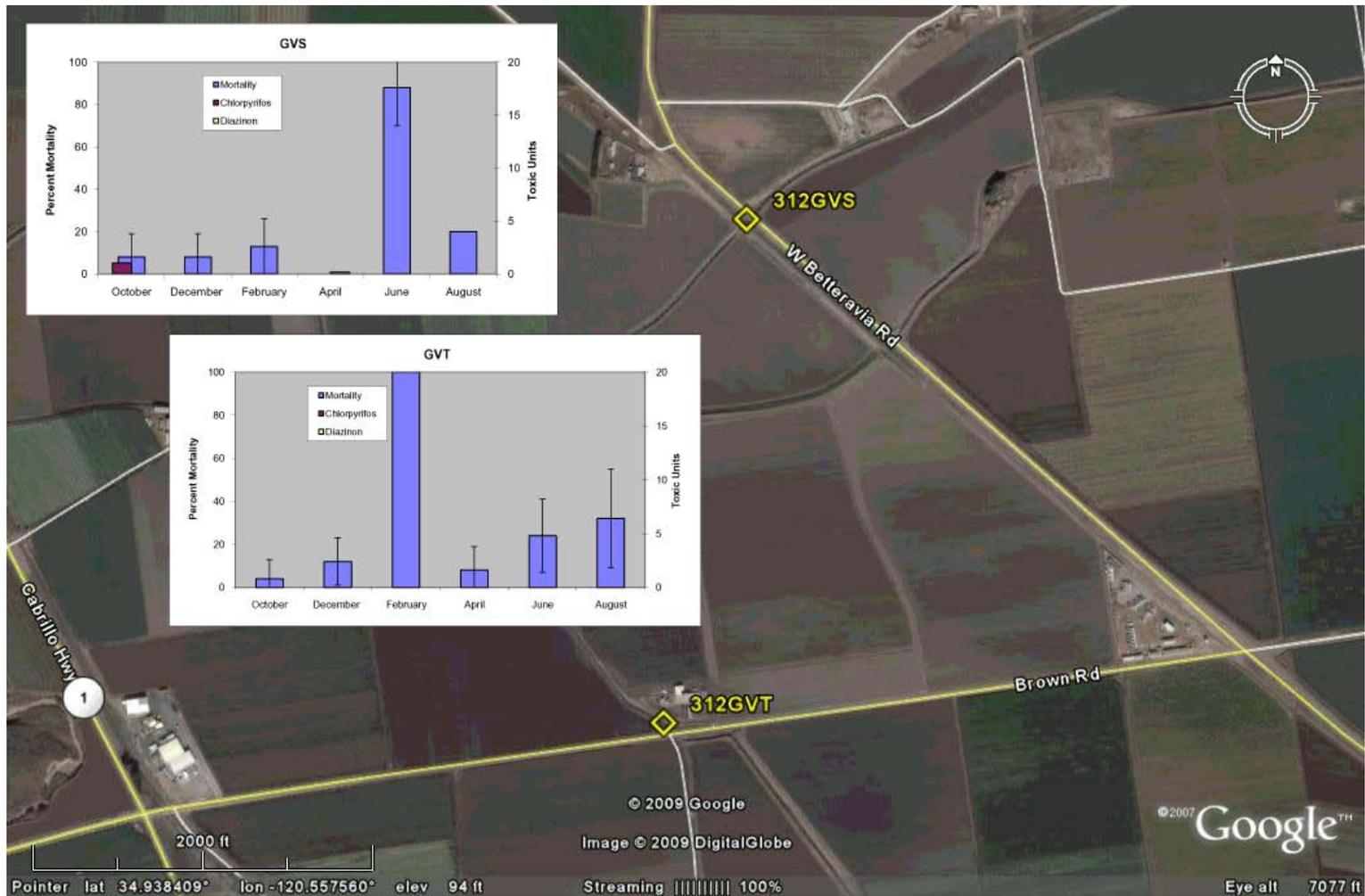


Figure 4. Google Earth© image of the Green Valley Creek area. Inset figures summarize water toxicity results (error bars indicate standard deviation) and ELISA measurements of chlorpyrifos and diazinon concentrations expressed in toxic units.

*Water Toxicity and Chemistry*

Three of the twelve samples collected at 312GVS and 312GVT were significantly toxic, but only one of the toxic samples had complete mortality. ELISA analyses did not detect chlorpyrifos or diazinon at high enough concentrations to cause the observed toxicity, but GC/MS analysis was conducted on the two partially toxic samples and detected approximately 0.5 TUs of chlorpyrifos in each (Figure 4, Table 11). Low concentrations of pyrethroid pesticides (ng/L) were detected and estimated in 312GVT. As discussed above, these concentrations were in the range of that would be toxic to *H. azteca* in water exposures.

Table 11. Green Valley Creek Area. Mean *C. dubia* survival (standard deviation), concentrations of chlorpyrifos and diazinon as measured by ELISA, and detected chemicals in select water samples as measured by GC/MS. All concentrations are in ng/L. Shading indicates significant toxicity or organophosphate pesticide concentrations greater than the *C. dubia* LC50. ND indicates non-detectable concentration. Bold values are below reporting limit, but above detection limit and are therefore estimated.

Date	312GVS			312GVT		
	Survival (SD)	Chlor.	Diaz.	Survival (SD)	Chlor.	Diaz.
10/23/2008	92 (11)	<b>55</b>	ND	96 (9)	ND	ND
12/5/2008	92 (11)	ND	ND	88 (11)	ND	ND
2/3/2009	87 (13)	ND	ND	0 (0)	ND	ND
4/1/2009	100 (0)	ND	ND	92 (11)	<b>58</b>	ND
6/12/2009	12 (18)	ND	ND	76 (17)	ND	ND
8/27/2009	80 (0)	ND	ND	68 (23)	ND	ND
<b>Detected Chemicals</b>	6/12/2009			8/27/2009		
<b>Organophosphates</b>	ng/L			ng/L		
Chlorpyrifos	31			19.4		
Diazinon	22.3			10.6		
Malathion	22.4			112.5		
<b>Pyrethroids</b>						
Cyfluthrin	ND			2.9		
Cypermethrin	ND			7.2		
Danitol	ND			<b>0.6</b>		
Esfenvalerate	ND			<b>1.1</b>		
Fenvalerate	ND			<b>1.5</b>		
Lambda-Cyhalothrin	ND			<b>1.6</b>		
Permethrin	ND			<b>7.9</b>		

*Water Toxicity Identification Evaluations*

The 312GVS sample from June 2009 was partially toxic (1.9 TUs) and was subjected to an abbreviated TIE (Table 12). No ELISAs were conducted during this TIE because ELISA results

in the initial test showed non-detects for chlorpyrifos and diazinon. Passing the sample through the HLB column completely removed toxicity, but the HLB eluate was not toxic. Similar results were observed with the Amberlite treatment. Addition of enzyme somewhat reduced toxicity, but because no pyrethroids were detected in the sample, it is likely that the reduction of toxicity was due to complexation of chemicals with the enzyme. Addition of PBO did not completely remove toxicity, but reduced toxicity below 1 TU. This result indicates that metabolically-activated organophosphates were contributing to toxicity. Additional chemical analysis with GC/MS detected 31 ng/L (0.6 TUs) of chlorpyrifos in the sample. This concentration would have been right at the detection limit for ELISA (30 ng/L), but detection of chlorpyrifos, as well as small amounts of diazinon and malathion, suggest that a mixture of organophosphate pesticides was causing toxicity in this sample.

Table 12. Green Valley Creek Area. Mean percent (standard deviation) survival of *C. dubia* in water toxicity identification evaluation (TIE) treatments. Toxic units are calculated from the dilution series by dividing the LC50 (in percent) by 100. Detected chemicals are listed with chemical LC50s for comparison. NA indicates not analyzed.

GVS (6/19/09) Treatment					Toxic Units	Other Detected Chemicals	Concentration ng/L	LC50 ng/L
	Control	25%	50%	100%				
Baseline	100 (0)	100 (0)	60 (20)	0 (0)	1.9	Chlorpyrifos	31	53
HLB	88 (11)	100 (0)	100 (0)	93 (12)	<1	Diazinon	22.3	320
HLB Eluate	100 (0)	89 (10)	100 (0)	100 (0)	<1	Malathion	22.4	2120
Amberlite	100 (0)	100 (0)	100 (0)	100 (0)	<1			
Amb. Eluate	93 (12)	100 (0)	93 (12)	94 (10)	<1			
Enzyme	93 (12)	100 (0)	100 (0)	40 (20)	1.1			
BSA	0 (0)*	0 (0)	0 (0)	20 (20)	NA			
PBO	87 (12)	100 (0)	87 (12)	47 (31)	<1			

### *Sediment Toxicity and Chemistry*

One sediment sample from each of the Green Valley sites was tested for toxicity and chemical analysis (Table 13). Station 312GVT was mildly toxic, but there were no detections of chlorpyrifos or diazinon in the interstitial water with ELISAs. A small amount of chlorpyrifos was detected in the 312GVS interstitial water, but the concentration was estimated because it was below the detection limit. GC/MS detected chlorpyrifos and a number of pyrethroid and organochlorine pesticides. The chlorpyrifos concentrations were greater than 0.5 TU and the sum TUs of the pyrethroids were 0.8 and 1.6 for 312GVS and 312GVT, respectively.

Permethrin contributed approximately one TU to 312GVT toxicity. Based on toxic unit analysis alone, pyrethroids were likely contributing to the observed toxicity in the 312GVT sample.

Table 13. Green Valley Creek Area. Mean *H. azteca* survival (standard deviation), interstitial water concentrations of chlorpyrifos and diazinon as measured by ELISA (ng/L), and detected chemicals in select samples as measured by GC/MS. Detected chemical concentrations are presented in total sediment concentrations (ng/g) and concentrations corrected for organic carbon content ( $\mu\text{g/g oc}$ ). Shading indicates significant toxicity or chemical concentrations greater than the *H. azteca* LC50. ND indicates non-detectable concentration. Bold values are below reporting limit, but above detection limit and are therefore estimated.

Date	312GVS			312GVT		
	Survival (SD)	Chlor.	Diaz.	Survival (SD)	Chlor.	Diaz.
6/19/2009	74 (30)	<b>54</b>	ND	64 (11)	ND	ND
<b>Detected Chemicals</b>	6/19/2009			6/19/2009		
<b>Organophosphates</b>	ng/g	$\mu\text{g/g oc}$		ng/g	$\mu\text{g/g oc}$	
Chlorpyrifos	17.9	1.19		15	0.99	
<b>Pyrethroids</b>						
Cyfluthrin	1.4	0.09		0.7	0.05	
Cypermethrin	3	0.20		1	0.07	
Esfenvalerate	1.4	0.09		3.6	0.24	
Fenvalerate	1.4			3.3		
L-Cyhalothrin	0.9	0.06		1.7	0.11	
Permethrin	ND			157.5	10.36	
<b>Organochlorines</b>						
2,4'-DDD	11.4			9		
2,4'-DDE	3.2			2.3		
4,4'-DDD	30.5			26.5		
4,4'-DDE	167			61.4		
4,4'-DDT	9.8			9.3		
Total DDT	221.9	14.79		108.5	7.14	
DCPA (Dacthal)	6.3			ND		
Dicofol	47.5			ND		
Dieldrin	10.5			ND		

### *Oso Flaco Creek Area*

#### *Area Description*

The Oso Flaco Watershed is located north of the Santa Maria River Estuary. Oso Flaco Creek (312OFC) is approximately 4 km upstream of the Oso Flaco Lake (312OFL) site.

*Water Toxicity and Chemistry*

Eight of twelve samples from these two sites were significantly toxic, and concentrations of chlorpyrifos and diazinon were all non-detects except for one measurement of chlorpyrifos in the August sample from 312OFC (Figure 5, Table 14). The detected concentration of chlorpyrifos was below the reporting limit, and therefore estimated. One toxic sample and one non-toxic sample were analyzed for additional chemicals with GC/MS. Several organophosphate and pyrethroid pesticides were detected, but only malathion was detected at a concentration greater than the toxicity threshold for *C. dubia*. Malathion was detected in the toxic sample from 312OFC. A TIE was attempted on the 312OFL sample from December 2008, but toxicity was not observed in the baseline sample of the TIE, so no conclusions were possible from this experiment.

Table 14. Oso Flaco Creek Area. Mean *C. dubia* survival (standard deviation), concentrations of chlorpyrifos and diazinon as measured by ELISA, and detected chemicals in select water samples as measured by GC/MS. All concentrations are in ng/L. Shading indicates significant toxicity or organophosphate pesticide concentrations greater than the *C. dubia* LC50. ND indicates non-detectable concentration. Bold values are below reporting limit, but above detection limit and are therefore estimated.

Date	312OFC			312OFL		
	Survival (SD)	Chlor.	Diaz.	Survival (SD)	Chlor.	Diaz.
10/23/2008	16 (17)	ND	ND	4 (9)	ND	ND
12/5/2008	78 (23)	ND	ND	4 (9)	ND	ND
2/3/2009	60 (24)	ND	ND	96 (9)	ND	ND
4/1/2009	96 (9)	ND	ND	0 (0)	ND	ND
6/12/2009	32 (33)	ND	ND	89 (10)	ND	ND
8/27/2009	0 (0)	<b>61</b>	ND	93 (10)	ND	ND
<b>Detected Chemicals</b>	6/12/2009			8/27/2009		
<b>Organophosphates</b>	ng/L			ng/L		
Chlorpyrifos	11.8			ND		
Dimethoate	ND			80.7		
Ethoprofos	11.9			6.4		
Malathion	2930			154.6		
<b>Pyrethroids</b>						
Cyfluthrin	ND			<b>0.9</b>		
Fluvalinate	ND			<b>0.6</b>		

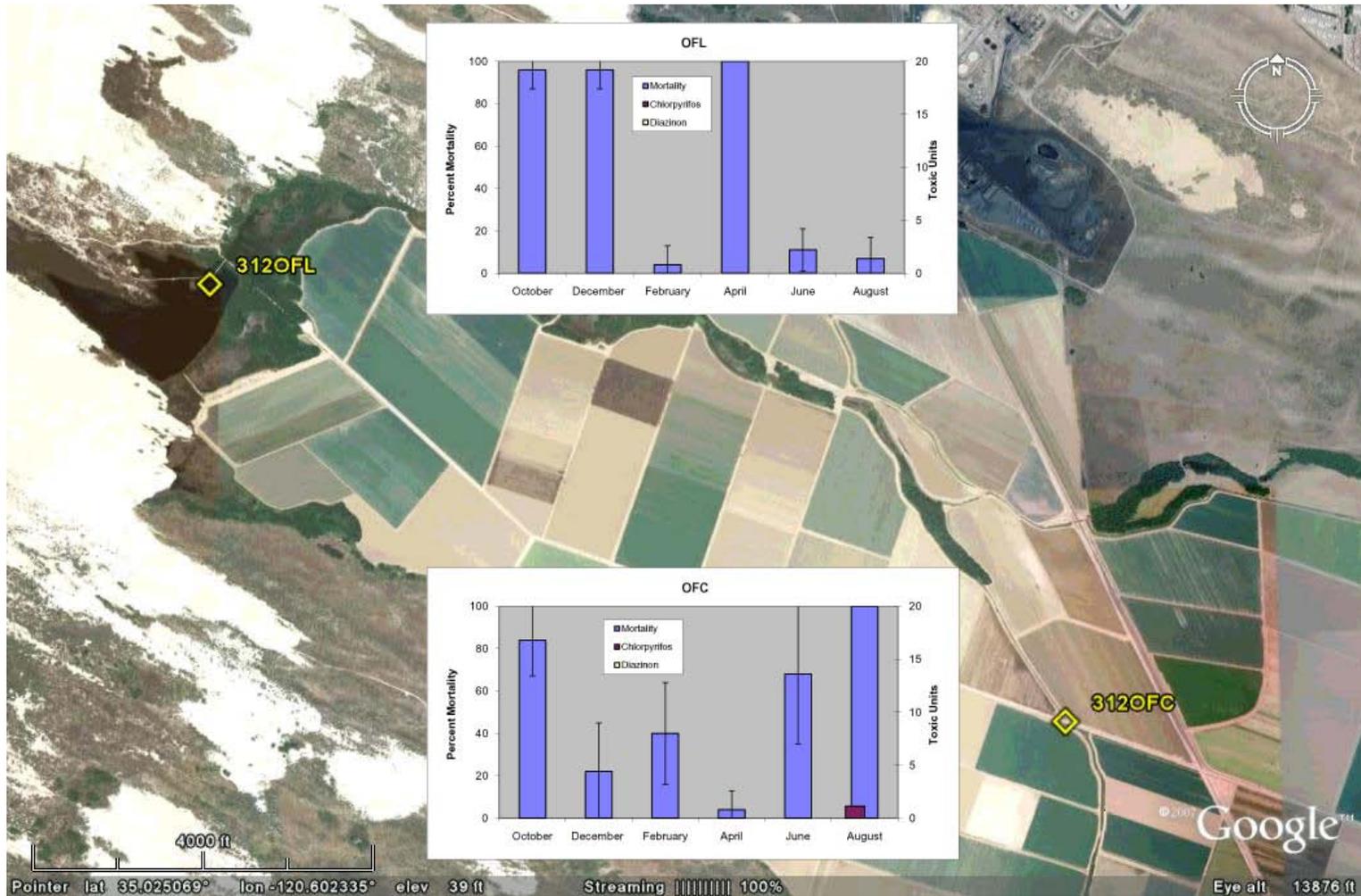


Figure 5. Google Earth© image of the Oso Flaco Creek area. Inset figures summarize water toxicity results (error bars indicate standard deviation) and ELISA measurements of chlorpyrifos and diazinon concentrations expressed in toxic units.

*Sediment Toxicity and Chemistry*

Sediment from 312OFC was tested once and sediment from 312OFL was tested three times for toxicity to *H. azteca*. Sediment from both sites was not significantly toxic and there was no detected chlorpyrifos or diazinon in the interstitial water (Table 15). Three of the four samples were analyzed for additional chemicals with GC/MS. Several pyrethroids (OFC, only) and organochlorine pesticides were detected, but none were above toxicity threshold concentrations for *H. azteca*.

Table 15. Oso Flaco Creek Area. Mean *H. azteca* survival (standard deviation), interstitial water concentrations of chlorpyrifos and diazinon as measured by ELISA (ng/L), and detected chemicals in select samples as measured by GC/MS. Detected chemical concentrations are presented in total sediment concentrations (ng/g) and concentrations corrected for organic carbon content ( $\mu\text{g/g oc}$ ). Shading indicates significant toxicity or chemical concentrations greater than the *H. azteca* LC50. ND indicates non-detectable concentration.

Date	312OFC			312OFL			
	Survival (SD)	Chlor.	Diaz.	Survival (SD)	Chlor.	Diaz.	
12/9/2008				79 (20)	ND	ND	
4/3/2009				94 (11)	ND	ND	
6/19/2009	79 (19)	ND	ND	95 (8)	ND	ND	
<b>Detected Chemicals</b>	6/19/2009			12/9/2008		6/19/2009	
<b>Pyrethroids</b>	ng/g	$\mu\text{g/g oc}$		ng/g	$\mu\text{g/g oc}$	ng/g	$\mu\text{g/g oc}$
Danitol	2.3			ND		ND	
Esfenvalerate	1.4	0.20		ND		ND	
Fenvalerate	0.6			ND		ND	
Permethrin	19.8	2.87		ND		ND	
<b>Organochlorines</b>							
2,4'-DDD	10.3			3.4		8.8	
2,4'-DDE	3			ND		2.3	
4,4'-DDD	25			7.7		17.4	
4,4'-DDE	198.5			66.1		145.5	
4,4'-DDT	6.2			2.4		6.9	
Total DDT	243	35.2		79.6	4.60	180.9	4.93
Dicofol	16.6			ND		37.6	
Dieldrin	ND			ND		11.6	
Toxaphene	ND			72.8		ND	

## *Santa Maria City Area*

### *Area Description*

Three stations were surveyed in the Santa Maria City area to determine the influence of urban inputs on water quality. The Bradley Channel originates on the eastern side of the city between Stowell Road and Betteravia Avenue. The first 2 km of the channel receive runoff from agricultural areas and a shopping center, but the next 3.5 km of the channel runs through primarily residential areas. The channel can also receive runoff from Highway 101 during storm events. The Bradley Channel station (312BRO) is immediately upstream of a recharge basin. Dry weather flow in Bradley Channel is primarily agricultural runoff with some residential trickle (Ellen Pritchett, City of Santa Maria, personal communication).

Blosser Channel flows for approximately 3 km on the western side of the city, and station 312BCD is sampled approximately 0.5 km from the river. This channel receives runoff from residential areas to the east and agricultural areas to the west, but is also connected to the Bradley Channel system. Flow in Bradley Channel can either pass through the recharge basin when it is full, or can be diverted to a channel that connects to a mid point in Blosser Channel (Rick Tomasini, Maintenance Superintendent, Santa Barbara County Flood Control District, personal communication). Station 312BCD is downstream of this confluence. During the study, Bradley Channel was generally flowing into the recharge basin, but in one sampling event water was being diverted to Blosser Channel. Blosser Channel tended to have very low flow, and in some cases it was difficult to observe any flow. Discharge in Blosser Channel was only measured on the day that water was diverted from Bradley Channel.

The Main Street Canal is an extension of the city's basin and canal system. Station 312MSS is located just downstream of where the canal daylight. This canal primarily receives urban and industrial inputs from sub-watersheds to the north and south of Main Street. The station itself is located next to agricultural field and might be influenced by overspray or direct discharges to the canal.

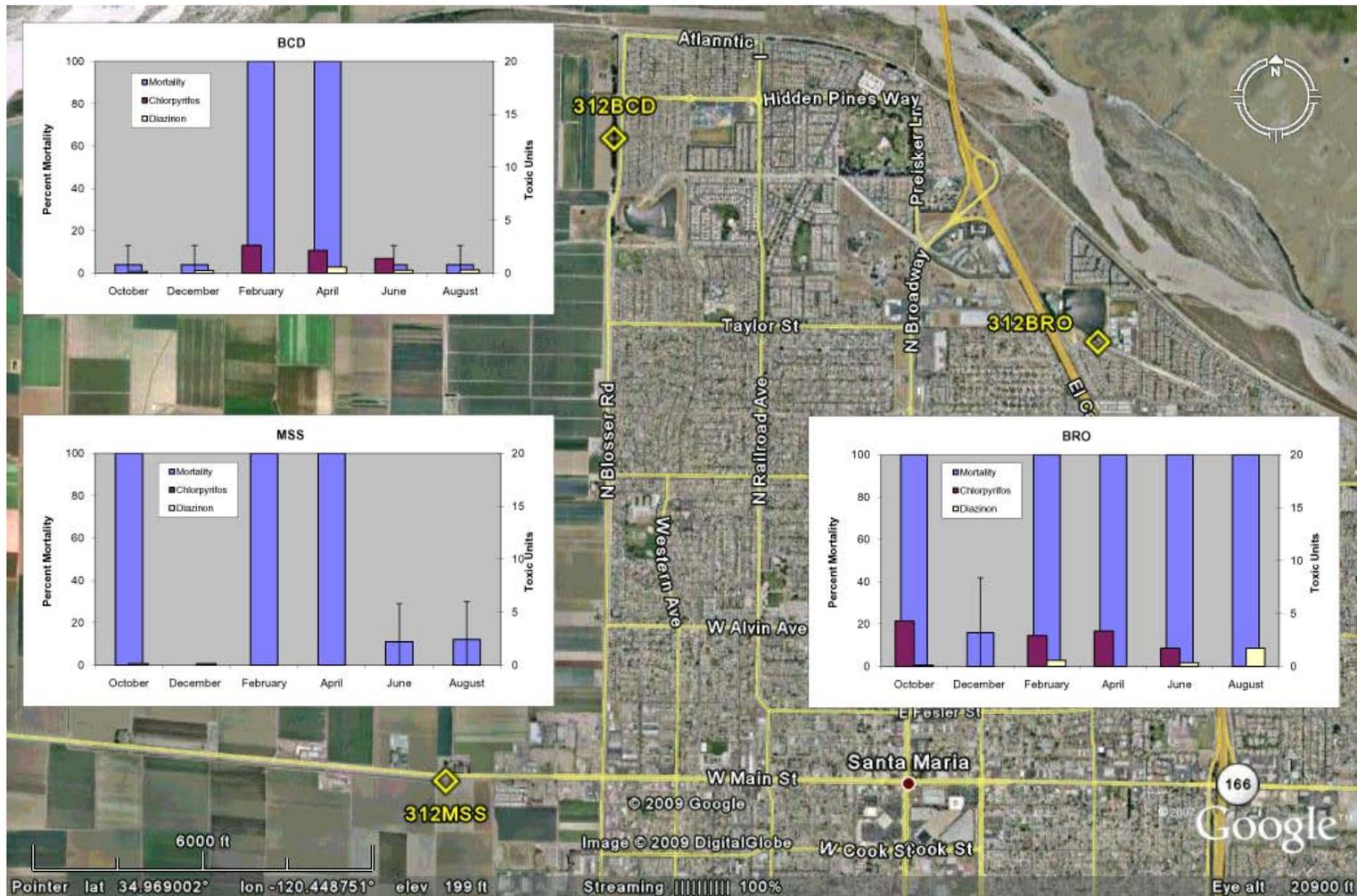


Figure 6. Google Earth© image of the Santa Maria City area. Inset figures summarize water toxicity results (error bars indicate standard deviation) and ELISA measurements of chlorpyrifos and diazinon concentrations expressed in toxic units.

Table 16. Santa Maria City Area. Mean *C. dubia* survival (standard deviation), concentrations of chlorpyrifos and diazinon as measured by ELISA, and detected chemicals in select water samples as measured by GC/MS. All concentrations are in ng/L. Shading indicates significant toxicity or organophosphate pesticide concentrations greater than the *C. dubia* LC50. ND indicates non-detectable concentration. Bold values are below reporting limit, but above detection limit and are therefore estimated.

Date	312BRO			312BCD			312MSS		
	Survival (SD)	Chlor.	Diaz.	Survival (SD)	Chlor.	Diaz.	Survival (SD)	Chlor.	Diaz.
10/23/2008	0 (0)	232	<b>41</b>	96 (9)	ND	<b>52</b>	0 (0)	ND	<b>51</b>
12/5/2008	84 (26)	ND	ND	96 (9)	ND	79	100 (0)	ND	<b>46</b>
2/3/2009	0 (0)	158	200	0 (0)*	141	ND	0 (0)	ND	ND
4/1/2009	0 (0)	180	ND	0 (0)	116	194	0 (0)	ND	ND
6/12/2009	0 (0)*	<b>92</b>	111	96 (9)	<b>74</b>	92	89 (18)	ND	ND
8/27/2009	0 (0)	ND	<b>572</b>	96 (9)	ND	102	88 (18)*	ND	ND
<b>Detected Chemicals</b>	6/12/2009			2/3/2009			8/27/2009		
<b>Organophosphates</b>	ng/L			ng/L			ng/L		
Chlorpyrifos	69.8			115			ND		
Diazinon	66.3			5.6			ND		
Dichlorvos	8.3			ND			ND		
Malathion	18.9			ND			ND		
<b>Pyrethroids</b>									
Bifenthrin	ND			3.6			<b>1.4</b>		
Cyfluthrin	ND			ND			5.5		
Cypermethrin	64.4			ND			18.3		
Danitol	ND			<b>0.7</b>			<b>1.4</b>		
Esfenvalerate	ND			<b>0.8</b>			ND		
Fenvalerate	ND			ND			<b>0.5</b>		
Fluvalinate	ND			<b>1</b>			ND		
Lambda-Cyhalothrin	ND			ND			4.3		
Permethrin	ND			ND			<b>14.6</b>		

### Water Toxicity and Chemistry

Station 312BRO was toxic five of the six times it was sampled and 312BCD was toxic two of six times (Figure 6, Table 16). Toxicity in all but one of these samples can be explained by concentrations of chlorpyrifos and diazinon as measured by ELISA. Additional chemical analysis of a toxic sample from each site detected concentrations of several organophosphate and pyrethroid pesticides, but chlorpyrifos was the only chemical that was detected at a concentration greater than the toxicity thresholds for *C. dubia*. Station 312MSS was toxic three of six times, but never contained toxic concentrations of chlorpyrifos or diazinon. Additional chemical analysis was conducted on a non-toxic sample from this site, and detected seven pyrethroid pesticides. Four of these pesticides were below the laboratory reporting limit, and three were well below the toxicity thresholds for *C. dubia*. Although these concentrations were below the

toxicity threshold for *C. dubia*, they are in the range of concentrations that would be toxic to *H. azteca* in water exposures.

#### *Water Toxicity Identification Evaluations*

Toxicity identification evaluations were conducted on water from 312BRO and 312BCD in June and February 2009, respectively. The 312BRO TIE was conducted on the sample that had low concentrations of organophosphate pesticides. Although chlorpyrifos was detected in this sample, the concentration was below the reporting limit and therefore ELISAs were not conducted on the TIE treatments. Station 312BRO had moderate toxicity that was removed by passing the sample through an HLB column and by adding Amberlite resin to the sample (Table 17), but column and resin eluates were not toxic. The enzyme and BSA treatments had poor blank survival and the results could not be interpreted. Addition of PBO removed toxicity, indicating organophosphate pesticides were causing toxicity, and GC/MS detected chlorpyrifos at a concentration higher than the *C. dubia* LC50.

Water from 312BCD was also moderately toxic with 2.1 TUs, and contained 2.2 TUs of chlorpyrifos, as measured by GC/MS. Passing the sample through an HLB column and adding Amberlite resin to the sample both removed toxicity, and the HLB eluate was toxic. Addition of PBO also completely removed toxicity, indicating that chlorpyrifos was the primary cause of toxicity. Although enzyme addition reduced toxicity, pyrethroid concentrations were very low. If pyrethroids were contributing to toxicity, addition of PBO would have increased the toxicity signal.

Table 17. Santa Maria City Area. Mean percent (standard deviation) survival of *C. dubia* in water toxicity identification evaluation (TIE) treatments. Toxic units are calculated from the dilution series by dividing the LC50 (in percent) by 100. ELISA chlorpyrifos concentrations and other detected chemicals are listed with chemical LC50s for comparison. Shading indicates concentrations that have exceeded the *C. dubia* LC50. NA indicates not analyzed. ND indicates not detected. \* indicates unacceptable blank survival.

312BRO (6/16/09) Treatment	Mean Percent (SD) Survival				Toxic Units	Other Detected Chemicals	Concentration ng/L	LC50 ng/L
	Control	25%	50%	100%				
Baseline	87 (23)	93 (12)	7 (12)	0 (0)	2.7	Chlorpyrifos	69.8	53
HLB Column	87 (23)	100 (0)	100 (0)	100 (0)	<1	Malathion	18.9	2120
HLB Eluate	80 (20)	93 (12)	87 (12)	60 (0)	<1	Cypermethrin	64.4	683
Amberlite	100 (0)	100 (0)	93 (12)	100 (0)	<1	Diazinon	66.3	320
Amb. Eluate	100 (0)	100 (0)	93 (12)	93 (12)	<1	Dichlorvos	8.3	130
Enzyme	7 (12)*	7 (12)	93 (12)	0 (0)	NA			
BSA	0 (0)*	0 (0)	0 (0)	0 (0)	NA			
PBO	93 (12)	87 (12)	93 (12)	73 (31)	<1			

312BCD (2/10/09) Treatment	Mean Percent (SD) Survival				Toxic Units	ELISA Chlor. ng/L	Other Detected Chemicals	Concentration ng/L	LC50 ng/L
	Control	25%	50%	100%					
Baseline	100 (0)	100 (0)	40 (20)	0 (0)	2.1	153	Chlorpyrifos	115	53
HLB Column	93 (12)	93 (12)	100 (0)	100 (0)	<1	ND	Bifenthrin	3.6	142
HLB Eluate	100 (0)	80 (0)	43 (6)	0 (0)	2.3	362	Danitol	0.7	
Amberlite	20 (20)*	100 (0)	100 (0)	93 (12)	<1	NA	Diazinon	5.6	320
Enzyme	33 (58)*	93 (12)	93 (12)	0 (0)	1.4	137	Esfenvalerate	0.8	
BSA	100 (0)	100 (0)	0 (0)	0 (0)	2.8	158	Fluvalinate	1	
PBO	76 (8)	93 (12)	100 (0)	93 (12)	<1	147			

### Sediment Toxicity and Chemistry

Stations from the Santa Maria City area were sampled once for sediment toxicity and chemistry. Significant toxicity was observed in all three samples, and chlorpyrifos and a number of pyrethroid and organochlorine pesticides were detected (Table 18). The concentration of chlorpyrifos in 312BCD was high enough to contribute to toxicity, but there were also several pyrethroid pesticides in all three samples exceeded *H. azteca* LC50s, and could have contributed to toxicity. Concentrations of organochlorine pesticides were well below toxic thresholds.

Table 18. Santa Maria City Area. Mean *H. azteca* survival (standard deviation), interstitial water concentrations of chlorpyrifos and diazinon as measured by ELISA (ng/L), and detected chemicals in select samples as measured by GC/MS. Detected chemical concentrations are presented in total sediment concentrations (ng/g) and concentrations corrected for organic carbon content ( $\mu\text{g/g oc}$ ). Shading indicates significant toxicity or chemical concentrations greater than the *H. azteca* LC50. ND indicates non-detectable concentration.

Date	312BRO			312BCD			312MSS		
	Survival (SD)	Chlor.	Diaz.	Survival (SD)	Chlor.	Diaz.	Survival (SD)	Chlor.	Diaz.
6/19/2009	6 (7)	75	64	0 (0)	144	76	0 (0)	ND	32
<b>Detected Chemicals</b>	6/19/2009			6/19/2009			6/19/2009		
<b>Organophosphates</b>	ng/g	µg/g oc		ng/g	µg/g oc		ng/g	µg/g oc	
Chlorpyrifos	15.2	0.46		224.7	2.91		9.8	0.29	
<b>Pyrethroids</b>									
Bifenthrin	83.1	2.51		374.6	4.86		21	0.61	
Cyfluthrin	ND			37.2	0.48		214	6.24	
Cypermethrin	35.8	1.08		58.2	0.75		499	14.55	
Danitol	4.3			139.2			13.7		
Esfenvalerate	ND			2.6	0.03		4.1	0.12	
Fenvalerate	ND			ND			4.1		
L-Cyhalothrin	1.5	0.05		15	0.19		125.2	3.65	
Permethrin	122.7	3.71		1279	16.59		539.9	15.74	
<b>Organochlorines</b>									
2,4'-DDD	4.1			19.2			22.5		
2,4'-DDE	ND			5.7			7.8		
4,4'-DDD	14.4			53.7			60.8		
4,4'-DDE	93.4			339			326		
4,4'-DDT	5.1			55.1			11.3		
Total DDT	117	3.53		473	6.13		428	12.48	
Chlordane-alpha	ND			7.1			7.6		
Chlordane-gamma	ND			5.2			6.9		
Dicofol	ND			738.4			ND		
Toxaphene	ND			ND			4.4		
trans-Nonachlor	ND			1.7			ND		

### *Sediment Toxicity Identification Evaluations*

All three sediment samples underwent TIEs to characterize the cause of toxicity. Toxicity of 312BRO sediment was significantly reduced with the addition of Amberlite resin, characterizing the cause of toxicity as an organic chemical. Addition of carboxylesterase enzyme reduced toxicity to a significantly greater extent than the addition of BSA, indicating the cause of toxicity was likely a pyrethroid pesticide. The addition of Amberlite resin and enzyme to the other two samples had no effect. Station 312BRO contained approximately 8 organic carbon-corrected TUs of pyrethroids, whereas 312BCD and 312MSS contained approximately 14 and 55 TUs of pyrethroids, respectively (Table 19). Station 312BCD also contained approximately 1.6 TUs of chlorpyrifos. It appears that the TIE treatments were able to reduce the pyrethroid toxicity in 312BRO, but were likely overwhelmed by the higher pyrethroid concentrations measured in 312BCD and 312MSS. These TIEs incorporated only a subset of treatments. For example no interstitial water TIEs were conducted and the whole sediment treatments were conducted only

on 100% sediment concentrations. This prevented resolution of toxicity, particularly in the samples contaminated by high concentrations of pyrethroids, such as BCD and MSS. Previous results have shown that TIEs of highly toxic samples are more effective when the procedures are applied to a dilution series of interstitial water and whole sediment. These procedures were not included in the current project due to lack of sufficient resources.

Table 19. Santa Maria City Area. Mean percent (standard deviation) survival of *H. azteca* in sediment toxicity identification evaluation (TIE) treatments, and detected organic carbon-corrected chemical concentrations that exceeded LC50 values (indicated by shading). \* indicates significant reduction of toxicity and significantly different from the dilution control. \*\* indicates significant reduction of toxicity and significantly different from BSA.

Treatment	312BRO Mean (SD)	312BCD Mean (SD)	312MSS Mean (SD)
Sample Baseline	4 (5)	0 (0)	0 (0)
10% Amberlite	26 (18)*	0 (0)	0 (0)
Control (10% Amberlite)	98 (4)	98 (4)	98 (4)
Enzyme	76 (15)**	12 (22)	0 (0)
Control (Enzyme)	100 (0)	100 (0)	100 (0)
BSA	14 (21)	0 (0)	0 (0)
Control (BSA)	100 (0)	100 (0)	100 (0)
PBO	0 (0)	0 (0)	0 (0)
Control (PBO)	96 (9)	96 (9)	96 (9)
Dilution Control	4 (5)	2 (4)	0 (0)
Control	98 (4)	98 (4)	98 (4)
Chemical	Organic Carbon-Corrected Concentrations ( $\mu\text{g/g OC}$ )		
Chlorpyrifos	0.46	2.91	0.29
Bifenthrin	2.51	4.86	0.61
Cypermethrin	1.08	0.75	14.55
L-Cyhalothrin	0.05	0.19	3.65
Permethrin	3.71	16.59	15.74

### ***Instantaneous Pesticide Loading Based on ELISA and GC/MS Measurements***

Discharge information was collected at eight of the ten stations (flow was not measured at 312SOL or 312OFL). Where possible, flow data were collected at each of the eight stations during four separate sampling events. In some cases there was measureable flow, but no detections of diazinon or chlorpyrifos with ELISA. In other cases there was no measureable flow. In these situations, loading was not calculated. A precipitation event took place nine days

before the March 31, 2009 sampling event. Approximately 0.8 cm of rain fell in the Santa Maria area on March 22, but it is unlikely this precipitation was having a significant effect on the flow measurements. All other flow events coincided with clear weather.

Table 20. Discharge measurements from four sample dates, and predicted daily loading of chlorpyrifos and diazinon based on ELISA measurements. ND indicates non-detectable concentration. Bold values are below reporting limit, but above detection limit and are therefore estimated.

Station	Source of Flow	Date	Discharge L/s	Chlorpyrifos g/day	Diazinon g/day
<b>312BCD</b>	Urban and Agriculture	3/31/09	15	0.15	0.25
<b>312BRO</b>	Urban and Agriculture	12/3/08	44	ND	ND
		6/10/09	6	<b>0.05</b>	0.06
<b>312GVS</b>	Agriculture	12/3/08	8	ND	ND
		3/31/09	22	ND	ND
		6/10/09	23	ND	ND
		8/26/09	45	ND	ND
<b>312GVT</b>	Agriculture	12/3/08	76	ND	ND
		3/31/09	24	<b>0.12</b>	ND
		6/10/09	16	ND	ND
		8/26/09	8	ND	ND
<b>312MSS</b>	Urban	12/3/08	2	ND	<b>0.01</b>
<b>312OFC</b>	Agriculture	12/3/08	55	ND	ND
		3/31/09	23	ND	ND
		6/10/09	45	ND	ND
		8/26/09	78	<b>0.41</b>	ND
<b>312ORC</b>	Agriculture	12/3/08	111	<b>0.49</b>	ND
		3/31/09	94	4.1	11.8
		6/10/09	198	ND	ND
		8/26/09	193	5.3	ND
<b>312SMA</b>	Agriculture	12/3/08	121	<b>0.70</b>	ND
		3/31/09	278	9.8	32.9
		6/10/09	185	<b>1.4</b>	<b>0.53</b>
		8/26/09	138	3.3	ND

Based on both ELISA analyses and GS/MS, there was insufficient data to allow a determination of temporal variability of loading of diazinon and chlorpyrifos at stations in the upper Santa Maria watersheds (Table 20 and Table 21). The relatively low diazinon and chlorpyrifos concentrations in water prevented calculations of loading at stations 312GVS, 312GVT, 312MSS, and 312OFC, based on ELISA measures. Loading for diazinon, chlorpyrifos or both pesticides was calculated on single sampling events for 312BRO (June 2009) and 312BCD (March 2009). Relatively low loadings (<0.5 g per day) of these pesticides were calculated for

the 312BRO and 312BCD stations, based on ELISA measures. Higher loadings were calculated for two stations in the lower Santa Maria watershed. Chlorpyrifos loading was 4.1 and 5.3 g/day, respectively, at 312ORC in March and June 2009, and diazinon loading was 11.8 grams per day at this station in March 2009. Chlorpyrifos loading was 9.8 and 3.3 g/day, respectively, at 312SMA in March and June 2009, and diazinon loading was 32.9 grams per day at this station in March 2009.

Table 21. Discharge measurements and predicted daily loading of detected chemicals in water. NA indicates “not analyzed” due to un-measurable flow. Bold values are predicted based on concentrations that were below the laboratory reporting limit. NA indicates not analyzed.

Measurement	312BRO 6/10/09	312GVS 6/10/09	312GVT 8/26/09	312MSS 8/26/09	312OFC 6/10/09	312ORC 8/26/09
<b>Discharge (L/s)</b>	6	23	8	NA	45	193
<b>Loading (g/d)</b>						
<b>Organophosphates</b>						
Chlorpyrifos	0.04	0.06	0.01		0.05	3.67
Diazinon	0.03	0.04	0.01			
Dichlorvos	0.004					
Dimethoate						0.133
Ethoprop					0.05	
Malathion	0.01	0.04	0.08		11.4	25.0
<b>Pyrethroids</b>						
Bifenthrin				NA		
Cyfluthrin			0.000002	NA		0.000035
Cypermethrin	0.03		0.005	NA		
Danitol			<b>0.0004</b>	NA		
Esfenvalerate			<b>0.0008</b>			
Fenvalerate			<b>0.001</b>	NA		
L-Cyhalothrin			<b>0.001</b>	NA		<b>0.012</b>
Permethrin			<b>0.005</b>	NA		
Resmethrin						<b>0.238</b>

Additional loadings were calculated based on GC/MS analyses (Table 21). These data show relatively low loadings of malathion at the 312BRO, 312GVT, and 312GVT stations (< 0.5 g per day), but considerably higher loadings of this pesticide at 312OFC (11.4 g per day), and at 312ORC (25 g per day). Loadings of a number of pyrethroid pesticides were also less than 0.5 g per day at the 312BRO, 312GVT, 312MSS, and 312ORC stations. However, given that pyrethroid pesticides are highly toxic at relatively low concentrations, particularly to amphipods, the lower pyrethroid loadings are likely toxicologically relevant.

While the chemical and discharge data are useful for providing a snapshot of relative chemical loadings at these stations, there is insufficient data to allow a thorough characterization of temporal and spatial patterns of chemical loading in this system. The chemical data used to calculate the loading presented in Table 21 is based on a single grab sample collected at the time the cross-sectional discharge was characterized at these stations. As such, the data likely do not reflect temporal variability in loadings at these stations. In addition, none of the discharge measures were taken during storm events, when loading would be expected to be highest. These data have been collected at the 312ORC station as part of the BMP project described above, but are not available for the current report. The loading characterization presented here is also limited by the number of GC/MS analyses collected for this project. There were too few GC/MS samples to allow a comprehensive characterization of loadings at these stations. Given their toxicological importance in the Santa Maria watershed, the analysis was particularly limited by the lack of pyrethroid analyses on a greater number of samples.

### ***Spatial and Temporal Patterns of Toxicity and Pesticide Concentrations***

In addition to data from the current TMDL project data from two other projects were reviewed to evaluate patterns of spatial and temporal toxicity and contamination. These included data from studies conducted by UC Davis for the State Water Resources Control Board (Best Management Practices – BMP), and data from the Central Coast Conditional Waiver Cooperative Monitoring Program (CMP). The current project sampled ten stations six times from October 2008 to August 2009. The BMP Project provides additional data dating back to January 2008, and the CMP project provides additional data back to August 2006. All references to CMP data are from three published reports (CCWQP, 2008, 2009, 2010) and personal communications with Sarah Greene (Central Coast Water Quality Preservation, Inc.). The BMP project provides additional data for station 312ORC and for a station in the Santa Maria Estuary that is approximately 0.5 km downstream of 312SMA. The CMP data adds to the temporal component for many of the stations and increases the spatial assessment by monitoring several upstream stations in the sub-watersheds. The BMP project was originally supposed to overlap with the sampling schedule of the current project, but due to the freezing of Consolidated Grants Proposition 51 funding, BMP sampling overlapped with the first two sampling events and continued at the termination of the current project.

*Spatial and Temporal Patterns of Toxicity and Pesticide Concentrations in the lower Santa Maria River and Green Valley Creek Areas*

The Green Valley Creek area is upstream of the Santa Maria River area. Water from this part of the watershed flows to the estuary via Orcutt Creek, and additional data from this area provides information on spatial patterns of pesticides of concern and toxicity. The greatest frequency of toxicity to *C. dubia* was observed in water samples collected from stations lowest in the Santa Maria watershed. Samples from the Santa Maria River area were toxic 83% of the time, whereas only 25% of samples from the Oso Flaco Creek area were toxic.

All of the samples from 312SMA were toxic in the current project, as were 60% of the samples from 312ORC. Similar results were observed in the fifteen BMP project sampling events between January 2008 and September 2009: 81% of the sample from the upper Santa Maria Estuary and 312ORC were toxic to *H. azteca* in water and 94% of samples were toxic to *C. dubia*. Both of these sites were periodically sampled by the CMP for toxicity and chemical analysis. From August 2006 to the present, 75% of samples from 312ORC were toxic and 80% of samples from 312SMA were toxic. Station 312SOL was also tested by the CMP between January and September 2008 and was toxic 75% of the time.

In many cases, water toxicity in the current project could be explained by concentrations of the organophosphate pesticides chlorpyrifos and diazinon. Concentrations of these pesticides were analyzed using ELISA and compared to their respective LC50s for *C. dubia*. Toxic units were calculated and summed based on the additive toxicity of these organophosphates. Based on the individual and combined TUs measured in these samples, there were sufficient concentrations of chlorpyrifos, diazinon, or both pesticides to account for the observed toxicity in half of the toxic samples. The TIE results support this conclusion and are discussed below. The highest incidence of elevated organophosphate concentrations occurred in samples from the Santa Maria River area, where there was the most toxicity. Elevated concentrations of chlorpyrifos and diazinon were also observed in 75% of the BMP project stations located in the Santa Maria River area. Organophosphate pesticides were analyzed in water from 312ORC and 312SMA during five CMP sampling events. Toxicity was observed in four of these events and in every case

chlorpyrifos concentrations were high enough to explain the observed toxicity. The data from the BMP project and the CMP demonstrate a consistent temporal pattern of water toxicity and organophosphate contamination for these stations.

In the current project, none of the toxic water samples from stations 312GVS and 312GVT contained sufficient concentrations of chlorpyrifos or diazinon to explain toxicity (as measured using ELISA). In addition, none of the other pesticides measured in water from these stations using GC/MS exceeded known toxicity thresholds for *C. dubia*. Station 312GVS, and an additional downstream station (312OR1), were also sampled extensively as part of the CMP. These two stations were toxic approximately 40% of the time. Unlike the current study, elevated concentrations of chlorpyrifos were measured in three of the four toxic samples that included synoptic chemical analysis. An additional upstream station on Green Valley Creek (312MAB) was toxic in one of two sampling events. The CMP also monitored three stations higher up in the Orcutt Creek watershed between January and September 2008 (312MHD, 312ORB and 312ORS). These stations were west of the town of Orcutt, and it is unclear whether they were significantly influenced by urban inputs. Together, these stations were sampled seven times, and were toxic twice. There were no chemical analyses conducted at these stations. In summary, the CMP data demonstrate consistent, but sporadic toxicity in the Green Valley Creek area, and a similar pattern of toxicity in the upper watershed

Sediment toxicity to the amphipod *H. azteca* was observed in samples from many of the stations which exhibited water toxicity. All sediment samples from the Santa Maria River area were toxic, but no sediment toxicity was observed at station 312GVS, and only moderate toxicity was observed at station 312GVT in the June 2009 sampling event. The BMP project monitored sediment toxicity three times at 312ORC and at eight stations in the Santa Maria River Estuary between June 2008 and October 2009. All of the samples from 312ORC were significantly toxic, as were most of the uppermost stations from the estuary. The CMP sampled 312ORC and 312SOL in April 2008 and 312 ORC and 312SMA in April 2009. Three of these four samples were significantly toxic. Station 312GVS was also sampled twice and was significantly toxic once. Station 312OR1 (downstream of 312GVS) was toxic in two sampling events. Three other samples were collected in the upper Orcutt Creek watershed (312MHD, 312ORB and 312ORS).

Station 312ORS was significantly toxic in two sampling events. Toxicity data from all of the projects demonstrate consistent low survival of *H. azteca* in test sediment in the Santa Maria River area and sporadic toxicity in the Green Valley Creek area and its upper watershed.

Based on comparisons to established toxicity thresholds, sediment toxicity in the Santa Maria River area was likely due to chlorpyrifos or mixtures of pyrethroids with chlorpyrifos. Interstitial water concentrations of chlorpyrifos in sediments from station 312SMA exceeded the *H. azteca* 10d LC50 in December 2008, and June 2009. The December 2008 sample from 312SMA contained a toxic concentration of the pyrethroid lambda-cyhalothrin, and this sample also contained cypermethrin and esfenvalerate (organic carbon-corrected concentrations). Sediment from 312ORC contained toxic concentrations of chlorpyrifos and cypermethrin. Two of the toxic 312ORC sediment samples and one of the toxic 312SMA sediment samples did not have any chemistry analyzed; therefore it is not possible to link the cause of toxicity to specific chemicals during these events. However, given the evidence from previous monitoring at these stations, toxicity here was likely caused by a combination of the same pesticides. The moderate toxicity observed in the June 2008 sample from 312GVT was likely due to mixtures of pyrethroids. There was no chemical analysis of samples from the CMP and sediment chemistry data from the BMP project is pending.

#### *Spatial and Temporal Patterns of Toxicity and Pesticide Concentrations in the Oso Flaco Creek Area*

In the current study, five of six samples from station 312OFC and three of six samples from station 312OFL were toxic, but none contained toxic concentrations of chlorpyrifos or diazinon. Concentrations of the organophosphate pesticide malathion exceeded the LC50 in one of the samples from 312OFC and this pesticide was detected in several other samples. The CMP sampled water from 312OFC twelve times between August 2006 and September 2009. Five of these samples were significantly toxic, and three of these toxic samples were accompanied by organophosphate pesticide analysis. One toxic sample contained approximately 3 TUs of chlorpyrifos, and another contained approximately 0.4 TUs of malathion. Although the CMP data show 312OFC to be toxic less frequently than the current study, the chemistry results are

similar with low detections of organophosphate pesticides. The possibility that malathion and other pesticides exerted toxicity through additive effects is discussed below.

The CMP also monitored toxicity at four upstream stations in the Oso Flaco Creek watershed, and one downstream tributary. The upstream stations (312BSR, 312OLR, 312OSR and 312USC) were sampled four times between January and September 2008, and the downstream tributary station (312OFN) was sampled ten times beginning in August 2006. Two of the upstream stations were located along a ditch that flows into the main creek just upstream of 312OFC. The other two stations were on the main stem of the creek. Collectively, these stations were toxic 63% of the time, but most of the toxic samples were from the creek stations. Organophosphate pesticides were not measured in the CMP samples from the creek, but analysis was conducted on four samples from station 312OFN. None of these samples were toxic or contained chlorpyrifos, diazinon or malathion. One sample from this station that did not have synoptic chemical analysis was toxic.

No sediments from stations 312OFC or 312OFL were toxic during the current study. No chlorpyrifos was detected in these sediments and only low concentrations of the pyrethroids esfenvalerate and permethrin were detected in 312OFC sediment. Four sediment samples were collected by the CMP in the upper watershed and the ditch adjacent to Oso Flaco Road; and all significantly toxic to *H. azteca*. The CMP samples were collected in April 2008, whereas the samples from the current project were collected between December 2008 and August 2009. No chemical analysis was conducted on the CMP samples.

#### *Spatial and Temporal Patterns of Water Toxicity and Pesticide Concentrations in the Santa Maria City Area*

The second highest incidence of organophosphate pesticide detection was in the Santa Maria City area where 60% of the water toxicity could be explained by concentrations of chlorpyrifos or diazinon that were greater than their respective toxicity thresholds. All of the toxic samples with high concentrations of organophosphate pesticides were from stations 312BCD and 312BRO. The CMP sampled an additional station upstream of 312BRO (312BCJ) ten times between August 2006 and September 2009. This station was toxic in every sampling event and

contained toxic concentrations of chlorpyrifos in the five events that were accompanied by chemical analysis. The concentrations of chlorpyrifos observed at 312BCJ were similar to those measured at the downstream stations in the current study: 312BRO and 312 BCD. Based on the location of 312BCJ, and the consistent chlorpyrifos-related toxicity that was observed, it is likely that this watershed is influenced more by agriculture than urban runoff.

One third of the water samples from 312MSS were toxic, but no (detected) pesticides were greater than toxicity thresholds. The CMP monitored toxicity at 312MSS twice in the summer of 2008, and monitored 312MSD (approximately 1.5 km downstream) ten times between August 2006 and September 2009. Both samples from 312MSS and half the samples from 312MSD were toxic to *C. dubia*. Three of the toxic 312MSD samples had synoptic chemical analysis and one sample contained toxic concentrations of chlorpyrifos. The other two samples contained approximately 0.25 TU of malathion. Station 312MSD receives urban flow from where the ditch daylights (at 312MSS), but this location could also be influenced by agricultural runoff.

Sediments from the City area were toxic in June 2008, the only time sediment toxicity was assessed at these stations. The CMP collected additional City area samples at 312BCJ and 312MSD in April 2009, and both of these samples were significantly toxic. Sediment from the City area stations had the highest concentrations of pyrethroid pesticides. Mixtures of toxic concentrations of the pyrethroid pesticides bifenthrin, cypermethrin, cyfluthrin, lambda-cyhalothrin and permethrin were measured in the samples from 312BCD, 312BRO and 312MSS in June 2009. In addition, chlorpyrifos in the 312BCD sediment sample from this date also exceeded the LC50 for *H. azteca*. No chemical analysis was conducted on the CMP samples.

### ***Evaluation of the Relative Contributions of Legacy and Current Use Pesticides to Water and Sediment Toxicity***

The weight of evidence from toxicity, chemistry and TIE data suggest that water toxicity to *C. dubia* in Santa Maria River and City areas was caused by organophosphate pesticides and not legacy organochlorine pesticides. Most of the observed toxicity in these areas was associated with elevated diazinon and chlorpyrifos. Malathion exceeded the LC50 for toxicity to *C. dubia*

in one sample from the Oso Flaco Creek area, but the cause of toxicity of other samples from this area and the Green Valley Creek area is unknown.

Many of the water samples analyzed in this study contained detectable concentrations of pyrethroids. While the majority of these samples did not exceed their respective LC50 values for pyrethroid toxicity to *C. dubia*, many of them exceeded the water LC50 values for toxicity to the amphipod *H. azteca*. Weston and Jackson (Weston and Jackson, 2009) found that LC50s for bifenthrin, cyfluthrin, and cypermethrin toxicity to *H. azteca* ranged from 1.7 to 3.3 ng/L. Water concentrations of pyrethroids measured at 312GVT, 312MSS, 312ORC, 312SOL and 312SMA exceeded this toxicity range. Cypermethrin in water was particularly high at 312GVT (7.2 ng/L), 312MSS (18.3 ng/L), and 312BRO (64.4 ng/L). These data suggest that in order to accurately characterize potential for water toxicity, both *C. dubia* and *H. azteca* should be tested (in water exposures) at sites where there is a potential for contamination from both organophosphate and pyrethroid pesticides.

Results of the water TIEs confirm that toxicity in the samples where TIEs were conducted was primarily due to organophosphate pesticides. Water TIEs were conducted on February 2009 samples from 312SOL, 312BCD and 312SMA. Additional TIEs were conducted on 312BRO and 312GVS using samples collected in June 2009. In three of the five water TIEs; toxicity was reduced by passing samples through an HLB solid-phase extraction column, which is designed to remove organic chemicals such as pesticides. In all of these samples, toxicity was observed in the column eluate treatment that was prepared from the solvent. All of these samples had chlorpyrifos concentrations above the LC50 and the chlorpyrifos was removed by the HLB columns. In all cases, toxic concentrations of chlorpyrifos were measured in the HLB column eluates. The concentration of chlorpyrifos in water from 312BRO was also greater than the LC50, but was below the reporting limit for ELISA. The HLB column eluate was not toxic in the 312BRO TIE, but because the concentration of chlorpyrifos was below the reporting limit, ELISAs were not conducted with the TIE. While diazinon and malathion were present in these four samples, their concentrations did not exceed their respective LC50s; however, it is possible some toxicity was contributed to these samples by diazinon and malathion through additivity. Water toxicity in the 312BCD and 312BRO TIEs was also significantly reduced with the

addition of the metabolic inhibitor piperonyl butoxide (PBO), which provides additional evidence that toxicity was caused by the metabolically-activated organophosphate pesticide chlorpyrifos.

Results of the 312GVS TIE were less conclusive. This sample contained 0.58 TUs of chlorpyrifos and low concentrations of diazinon and malathion. Toxicity was removed when the sample was passed through the HLB column, but the HLB eluate was not toxic. Toxicity of this sample was also reduced (but not removed) with addition of the metabolic inhibitor PBO. These results suggest toxicity was caused partially by chlorpyrifos, but that some other chemical was present at toxic concentrations. Toxicity was also reduced with addition of the carboxylesterase enzyme, which suggests toxicity was partially due to a pyrethroid pesticide. No pyrethroid was detected in this water, however.

The results of the water TIEs were somewhat constrained by the design of the TIEs. Due to limited resources, this project was designed to use abbreviated TIEs which did not include all treatments. In some cases this may have limited the ability to resolve toxicity due to pesticide mixtures. For example, in situations where pyrethroids and organophosphates co-occur at toxic concentrations we have found it to be useful to include additional treatments to help resolve toxicity due to these pesticide classes. These include temperature manipulations, addition of PBO and esterase enzyme in combination, and GC/MS analyses of column eluates. These treatments were not included in order to allow a greater number of TIEs to be conducted on toxic samples from all stations. We also note that we attempted to conduct a TIE on a toxic sample from Oso Flaco Lake. While this TIE was conducted on a sample that had shown high mortality in the initial test, this TIE was not successful because no toxicity was observed in the baseline (un-manipulated) sample during the TIE.

Sediment samples from the 312SMA, 312ORC, 312BCD, 312BRO and 312MSS were selected for sediment TIEs. When compared to the chemical analyses of these samples, the TIE results suggests toxicity of all of these samples was due to either chlorpyrifos, a number of pyrethroids, or chlorpyrifos and pyrethroids in combination. While a number of organochlorine pesticides were also detected in these samples, none of these exceeded known toxicity thresholds or

guideline values. The TIE results show that toxicity of three of the samples (312SMA, 312ORC, and 312BRO) was reduced with the addition of the Amberlite resin. Amberlite binds organic chemicals such as pesticides, so reductions in toxicity with resin addition suggests toxicity was caused, in part, by organic chemicals. Toxicity of 312SMA, 312BCD, and 312BRO were also partially removed with addition of the carboxylesterase enzyme to the sediment overlying water. This suggests toxicity was partially due to pyrethroid pesticides. Concentrations of chlorpyrifos in 312SMA, 312ORC, and 312BCD sediments exceeded the organic carbon-corrected LC50 for chlorpyrifos toxicity to *H. azteca*. In addition, samples from each of the stations contained at least two pyrethroids at concentrations exceeding their respective organic carbon-corrected LC50 values. Cypermethrin was detected at toxic concentrations at four of the five stations, and samples from 312BCD and 312MSS contained four pyrethroids at toxic concentrations.

As with the water TIEs discussed above, the sediment TIEs were somewhat constrained by the lack of additional treatments (e.g., no temperature manipulations). In addition, because the TIE samples were highly toxic (survival range = 0 – 4%), many of the TIE treatments were likely less effective at reducing toxicity. This problem is overcome by testing each TIE treatment using progressively lower dilutions of the sediment sample. This was not possible in these experiments due to the use of abbreviated TIEs as a means to allow a greater number of TIEs.

It should also be noted that despite evidence suggesting that legacy pesticides are likely not responsible for the acute water and sediment toxicity observed during this study, it is possible these pesticides impact biota in these systems via chronic exposure to pesticides. For example, some 303(d) listings in these watersheds are based partly on results of early TSM program reports of elevated organochlorine pesticides in fish collected from the lower Santa Maria River. Analyses of fish and sand crab tissues as part of recent monitoring for the BMP project (described above) will allow us to determine the degree to which these pesticides continue to bioaccumulate in fish and invertebrates in the Santa Maria estuary and its vicinity.

***Relative Chemical Contamination and Toxicity at Urban, Agriculture, and Mixed Land-Use Stations.***

This study was intended to compare relative contamination and toxicity at stations influenced primarily by agriculture inputs, to those influenced primarily by urban inputs. Three stations (312BRO, 312BCD, and 312MSS) were designated as urban-influenced stations. As mentioned above, 312BRO and 312BCD are both influenced by agriculture tailwater inputs, particularly during dry weather.

Recent conversations with Ellen Pritchett of the City of Santa Maria suggest that of these three stations, only 312MSS can be classified as a primarily urban-influenced station, and this station is directly adjacent to fields. In addition to urban inputs, this station may also receive some pesticide inputs via overspray and storm runoff (Ellen Pritchett, City of Santa Maria, personal communication). Station 312BRO receives agriculture runoff from fields east of this station. In addition, water from 312BRO flows to the 312BCD station after either passing through a recharge basin, or directly through a bypass. Both 312BRO and 312BCD therefore are likely influenced by mixtures of urban and agriculture inputs. This is reflected in the pesticides present in water and sediment at these stations.

Toxic concentrations of diazinon, chlorpyrifos, or mixtures of both pesticides were detected in water from all Santa Maria watershed stations except 312GVS, 312GVT, and 312MSS. Chlorpyrifos was detected in sediments from all Santa Maria watershed stations, including 312MSS. Since diazinon and chlorpyrifos are no longer available for residential use, the presence of these pesticides at 312BCD and 312BRO indicate agriculture inputs affect these stations. It should be noted that the lack of diazinon and chlorpyrifos in water at 312MSS and the considerably lower concentrations of chlorpyrifos in 312MSS sediment likely reflect that this station is less impacted by agricultural inputs. Pyrethroids were found at all stations in the Santa Maria River watershed, but not in the Oso Flaco Creek watershed. The highest concentrations of pyrethroids were detected in the Santa Maria urban-influenced stations. Bifenthrin, cypermethrin and cyfluthrin were found at higher concentrations in sediments at 312BCD, 312BRO, and 312MSS. These pyrethroids have been associated with urban inputs in previous

studies (Holmes et al., 2008), and likely indicate that these stations receive urban as well as agricultural inputs.

The stations with the greatest toxicity and contamination in this study were those in the lower Santa Maria River area (312SOL and 312SMA). These stations receive predominately agricultural inputs via agriculture tailwater runoff. Our ability to differentiate between relative contamination and toxicity at urban versus agricultural stations is constrained by the fact that all of these stations are likely influenced by agricultural inputs. It should also be noted that the two agricultural stations in the Green Valley Creek area (312GVS and 312GVT) also demonstrated lower toxicity and chemical contamination. The toxicity of these stations could not be explained simply by the concentrations of the measured analytes, but the TIE conducted on 312GVS suggests the additive toxicity of organophosphates were contributing to toxicity.

#### ***Assessing the potential for additive toxicity due to multiple contaminants***

As discussed above, the majority of the water and sediment samples from these stations were contaminated by mixtures of pesticides. Water from six of the ten stations contained mixtures of diazinon and chlorpyrifos, and toxicity of these pesticides have been shown to be additive to *C. dubia* (Bailey et al., 1997). In addition, samples from many of these stations also contained mixtures of pyrethroid pesticides in water and/or sediment. Pyrethroid toxicity has also been demonstrated to be additive to the amphipod *H. azteca* (Weston and Jackson, 2009). In many cases, water samples were also contaminated with other organophosphate pesticides, such as malathion. The potential for additive toxicity of mixtures of malathion, diazinon and chlorpyrifos has not been reported, but since all are neurotoxins (acetylcholinesterase inhibitors), the possibility exists that diazinon and chlorpyrifos toxicity is increased in the presence of malathion (Norberg-King et al., 1991). Recent research has also demonstrated that toxicity of certain organophosphate pesticides increases in the presence of pyrethroid pesticides (e.g., (Denton et al., 2003)). For example, Denton et al. (2003) found greater than additive toxicity of diazinon to fathead minnows in the presence of the pyrethroid esfenvalerate.

## Summary and Conclusion

The current study demonstrated through toxicity testing, chemical analyses, and TIEs that water toxicity to *C. dubia* was caused primarily by organophosphate pesticides, and sediment toxicity to *H. azteca* was caused primarily by chlorpyrifos and pyrethroid pesticides. Up to 75% of toxicity could be explained by chlorpyrifos and diazinon concentrations in the lower Santa Maria River and City areas (Table 22). Although some toxicity occurred in the Oso Flaco Creek and Green Valley Creek areas, most organophosphate pesticides were not detected in these samples. Sediment toxicity followed a similar pattern with the most severe toxicity observed in the Santa Maria River and City areas (Table 23). Toxicity in these areas was caused by a combination of chlorpyrifos and pyrethroid pesticides. The highest concentrations of pyrethroids were detected at stations with urban influence.

The results from the current study corroborate results of the concurrent BMP and CMP studies in the Santa Maria River and Oso Flaco Creek watersheds. These studies demonstrate that toxicity is caused by mixtures of organophosphate and pyrethroid pesticides. Previous RWQCB studies in these watersheds have found similar results. Recent studies also showed that water and sediment toxicity at many of the same stations studies in the current projects was due to mixtures of chlorpyrifos, diazinon, and pyrethroid pesticides (Anderson et al., 2006; Phillips et al., 2006). These studies have also shown ecological impacts of pesticides in the Santa Maria River and the Santa Maria River estuary, including impacts on benthic macroinvertebrate communities.

One concern in the current study is identification of high incidences of water toxicity in Oso Flaco Creek and Lake. Oso Flaco Lake is considered one of the most ecologically important water bodies in this area. The current results did not allow identification of the causes of this toxicity. Future studies in Oso Flaco Lake should include more detailed toxicity identification evaluations, combined with comprehensive chemical analyses of water. This approach could also be used in the Green Valley Creek area, although there were fewer toxic samples at these stations.

Table 22. Summary of *C. dubia* percent survival (standard deviation), concentrations of chlorpyrifos and diazinon as measured by ELISA, and detected chemicals as measured by GC/MS. Shading indicates significant toxicity or organophosphate pesticide concentrations greater than the *C. dubia* LC50. Bolded chemical concentrations were measured below the laboratory reporting limit, and are therefore estimated.

Sample	10/23/2008			12/5/2008			2/3/2009			4/1/2009			6/12/2009			8/27/2009			Elevated OP TU in Toxic Samples
	Survival Mean (SD)	Chlor. ng/L	Diaz. ng/L	Survival Mean (SD)	Chlor. ng/L	Diaz.	Survival Mean (SD)	Chlor. ng/L	Diaz. ng/L										
<b>312SOL</b>	0 (0)	361	1310	0 (0)	491	ND	0 (0)	1013	73	0 (0)	692	1832	0 (0)	<b>55</b>	<b>41</b>	0 (0)	371	ND	5 of 6
<b>312ORC</b>				92 (18)	<b>51</b>	ND	0 (0)	921	<b>43</b>	0 (0)	508	1458	88 (11)	ND	ND	0 (0)	317	ND	3 of 3
<b>312SMA</b>	0 (0)	241	1384	64 (26)	<b>67</b>	ND	0 (0)	897	<b>48</b>	0 (0)	410	1368	0 (0)	<b>88</b>	<b>33</b>	0 (0)	276	ND	4 of 6
<b>312GVS</b>	92 (11)	<b>55</b>	ND	92 (11)	ND	ND	87 (13)	ND	ND	100 (0)	ND	ND	12 (18)	ND	ND	80 (0)	ND	ND	0 of 1
<b>312GVT</b>	96 (9)	ND	ND	88 (11)	ND	ND	0 (0)	ND	ND	92 (11)	<b>58</b>	ND	76 (17)	ND	ND	68 (23)	ND	ND	0 of 2
<b>312OFC</b>	16 (17)	ND	ND	78 (23)	ND	ND	60 (24)	ND	ND	96 (9)	ND	ND	32 (33)	ND	ND	0 (0)	<b>61</b>	ND	0 of 5
<b>312OFL</b>	4 (9)	ND	ND	4 (9)	ND	ND	96 (9)	ND	ND	0 (0)	ND	ND	89 (10)	ND	ND	93 (10)	ND	ND	0 of 3
<b>312BRO</b>	0 (0)	232	<b>41</b>	84 (26)	ND	ND	0 (0)	158	200	0 (0)	180	ND	0 (0)	<b>92</b>	111	0 (0)	ND	<b>572</b>	4 of 5
<b>312BCD</b>	96 (9)	ND	<b>52</b>	96 (9)	ND	79	0 (0)	141	ND	0 (0)	116	194	96 (9)	<b>74</b>	92	96 (9)	ND	102	2 of 2
<b>312MSS</b>	0 (0)	ND	<b>51</b>	100 (0)	ND	<b>46</b>	0 (0)	ND	ND	0 (0)	ND	ND	89 (18)	ND	ND	88 (18)	ND	ND	0 of 3
Number of Toxic Samples	6			4			8			7			5			6			

Table 22 Continued

Sample	Mean Percent Survival (SD)	Sample Date	Chlorpyrifos	Diazinon	Dichlorvos	Dimethoate	Ethoprofos	Malathion	Bifenthrin	Cyfluthrin	Cyhalothrin	Cypermethrin	Danitol	Esfenvalerate	Fenvalerate	Fluvalinate	Permethrin
<b>312BRO</b>	0 (0)	2/3/09	1874	20.8	ND	ND	ND	ND	ND	ND	<b>1.9</b>	4.2	ND	<b>1.9</b>	<b>0.9</b>	ND	ND
<b>312BCD</b>	96 (9)	8/27/09	220	ND	ND	8	ND	1497	ND	2.1	<b>0.7</b>	ND	ND	ND	ND	ND	ND
<b>312MSS</b>	0 (0)	2/3/09	1133	20.7	ND	ND	ND	19.6	ND	ND	2.1	3.5	ND	<b>1.2</b>	<b>0.8</b>	<b>0.5</b>	ND
<b>312GVS</b>	12 (18)	6/12/09	31	22.3	ND	ND	ND	22.4	ND	ND	ND	ND	ND	ND	ND	ND	ND
<b>312GVT</b>	68 (23)	8/27/09	19.4	10.6	ND	ND	ND	113	ND	2.9	<b>1.6</b>	7.2	<b>0.6</b>	<b>1.1</b>	<b>1.5</b>	ND	<b>7.9</b>
<b>312OFC</b>	32 (33)	6/12/09	11.8	ND	ND	ND	11.9	2930	ND	ND	ND	ND	ND	ND	ND	ND	ND
<b>312OFL</b>	93 (10)	8/27/09	ND	ND	ND	80.7	6.4	155	ND	<b>0.9</b>	ND	ND	ND	ND	ND	<b>0.6</b>	ND
<b>312SOL</b>	0 (0)	6/12/09	69.8	66.3	8.3	ND	ND	18.9	ND	ND	ND	64.4	ND	ND	ND	ND	ND
<b>312ORC</b>	0 (0)	2/3/09	115	5.6	ND	ND	ND	ND	3.6	ND	ND	ND	<b>0.7</b>	<b>0.8</b>	ND	<b>1</b>	ND
<b>312SMA</b>	0 (0)	8/27/09	ND	ND	ND	ND	ND	ND	<b>1.4</b>	5.5	4.3	18.3	<b>1.4</b>	ND	<b>0.5</b>	ND	<b>14.6</b>

Table 23. Summary of *H. azteca* percent survival (standard deviation), concentrations of chlorpyrifos and diazinon as measured by ELISA in sediment interstitial water, and concentrations of detected chemicals in sediments as measured by GC/MS. Shading indicates significant toxicity and chemical concentrations greater than the *H. azteca* LC50. \* indicates organic carbon-corrected concentration greater than LC50. Bold concentrations are below reporting limits and are therefore estimated.

Station	SOL	ORC	ORC	ORC	SMA	SMA	SMA	GVS	GVT	OFC	OFL	OFL	OFL	BRO	BCD	MSS
Analysis Date	6/19/09	12/9/08	4/3/09	6/19/09	12/9/08	4/3/09	6/19/09	6/19/09	6/19/09	6/19/09	12/9/08	4/3/09	6/19/09	6/19/09	6/19/09	6/19/09
Survival (SD)	5 (5)	3 (7)	8 (14)	3 (5)	0 (0)	0 (0)	5 (8)	74 (30)	64 (11)	79 (19)	79 (20)	94 (11)	95 (8)	6 (7)	0 (0)	0 (0)
Interstitial Chlorpyrifos (ng/L)	173	134	325	387	721	589	108	<b>54</b>	ND	ND	ND	ND	ND	<b>75</b>	144	ND
Interstitial Diazinon (ng/L)	<b>41</b>	ND	946	<b>52</b>	<b>53</b>	491	<b>39</b>	ND	ND	ND	ND	ND	ND	64	76	<b>32</b>
<b>Detected Chemicals in Sediment (ng/g)</b>																
Chlorpyrifos	41.4*	38.5*	NA	NA	164.4*	NA	58.9*	17.9	15	ND	ND	NA	ND	15.2	224.7	9.8
Bifenthrin	ND	ND	NA	NA	ND	NA	ND	ND	ND	ND	ND	NA	ND	83.1*	375*	21*
Cyfluthrin	ND	ND	NA	NA	ND	NA	ND	1.4	0.7	ND	ND	NA	ND	ND	37.2	214*
Cyhalothrin	1.4	2.9	NA	NA	14.1*	NA	2.4	0.9	1.7	ND	ND	NA	ND	1.5	15	125*
Cypermethrin	2.3	3.5*	NA	NA	6.2	NA	11	3	1	ND	ND	NA	ND	35.8*	58.2*	499*
Danitol	ND	ND	NA	NA	ND	NA	ND	ND	ND	2.3	ND	NA	ND	4.3	139.2	13.7
Esfenvalerate	1.6	2.8	NA	NA	5.5	NA	2.8	1.4	3.6	1.4	ND	NA	ND	ND	2.6	4.1
Fenvalerate	0.9	1.2	NA	NA	2.4	NA	2.4	1.4	3.3	0.6	ND	NA	ND	ND	ND	4.1
Permethrin	ND	ND	NA	NA	ND	NA	ND	ND	157.5	19.8	ND	NA	ND	122.7	1279*	540*
2,4'-DDD	18	10.3	NA	NA	29.5	NA	20	11.4	9	10.3	3.4	NA	8.8	4.1	19.2	22.5
2,4'-DDE	4.4	2.5	NA	NA	6.5	NA	6.1	3.2	2.3	3	ND	NA	2.3	ND	5.7	7.8
2,4'-DDT	ND	ND	NA	NA	3	NA	ND	ND	ND	ND	ND	NA	ND	ND	ND	ND
4,4'-DDD	32.6	21	NA	NA	68.9	NA	31.6	30.5	26.5	25	7.7	NA	17.4	14.4	53.7	60.8
4,4'-DDE	207.5	130.1	NA	NA	337.6	NA	275.3	167	61.4	198.5	66.1	NA	146	93.4	339	326
4,4'-DDT	5.8	3.2	NA	NA	10.1	NA	7.6	9.8	9.3	6.2	2.4	NA	6.9	5.1	55.1	11.3
Total DDT	268.3	167.1	NA	NA	455.6	NA	340.6	221.9	108.5	243	79.6	NA	181	117	473	428
Chlordane-alpha	ND	ND	NA	NA	ND	NA	ND	ND	ND	ND	ND	NA	ND	ND	7.1	7.6
Chlordane-gamma	ND	ND	NA	NA	ND	NA	ND	ND	ND	ND	ND	NA	ND	ND	5.2	6.9
DCPA (Dacthal)	ND	ND	NA	NA	ND	NA	ND	6.3	ND	ND	ND	NA	ND	ND	ND	ND
Dicofol	48.8	ND	NA	NA	10.3	NA	61.4	47.5	ND	16.6	ND	NA	37.6	ND	738.4	ND
Dieldrin	ND	ND	NA	NA	ND	NA	ND	10.5	ND	ND	ND	NA	11.6	ND	ND	ND
Toxaphene	ND	88.63	NA	NA	551.92	NA	ND	ND	ND	ND	72.8	NA	ND	ND	ND	4.4
trans-Nonachlor	ND	ND	NA	NA	ND	NA	ND	ND	ND	ND	ND	NA	ND	ND	1.7	ND

Elevated concentrations of organophosphates were detected at stations with mixed urban and agricultural inputs. Although chlorpyrifos and diazinon are no longer available for public or residential use, it is possible that residents proximate to these sites are using residual stocks of these chemicals or they are being used by commercial applicators. Further research is needed to confirm the sources of these organophosphates. Source detection could be accomplished by measuring discharge and organophosphate concentrations in upstream reaches that only receive agriculture inputs, then comparing these results to discharge characterizations and chemical concentrations in isolated inputs from the urban areas.

There are a number of management practices available that have been shown to be effective at reducing run-off of the pesticides identified in the current study. Recent studies in the Salinas Valley and elsewhere have demonstrated the utility of integrated vegetated treatment systems (VTS) for removing pesticides causing water and sediment toxicity (Anderson et al., 2008; Hunt et al., 2008). Vegetated systems that incorporate a combination of grass-lined ditches, settling ponds, and enzyme treatment (Landguard®) have been shown to completely remove water toxicity associated with organophosphate pesticides, and to greatly reduce loadings of pyrethroid and organochlorine pesticides. While aspects of these practices are the subject of continuing discussions between the different stakeholders, a growing body of scientific evidence suggests these methods hold promise for reducing toxic concentrations in Central Coast watersheds. Once Regional Board policy is formalized for the use of these practices, these systems should prove useful in addressing pesticide loading issues in the Santa Maria and Oso Flaco Creek watersheds as the TMDL process proceeds. As management practices are implemented, then downstream stations should be monitored to measure their effectiveness.

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## Appendix

Table A1. Chemicals analyzed in water and sediment.

Analyte	Sediment	Water	Analyte	Sediment
<b>Organophosphate Pesticides</b>			<b>Organochlorine Pesticides</b>	
Azinphos Methyl	x	x	2,4'-DDD	x
Bolstar (Sulprofos)	x	x	2,4'-DDE	x
Chlorpyrifos	x	x	2,4'-DDT	x
Demeton	x	x	4,4'-DDD	x
Diazinon	x	x	4,4'-DDE	x
Dichlorvos	x	x	4,4'-DDT	x
Dimethoate	x	x	Aldrin	x
Disulfoton	x	x	BHC-alpha	x
Ethoprop (Ethoprofos)	x	x	BHC-beta	x
Ethyl Parathion	x	x	BHC-delta	x
Fenchlorphos (Ronnel)	x	x	BHC-gamma	x
Fenitrothion	x	x	Chlordane-alpha	x
Fensulfothion	x	x	Chlordane-gamma	x
Fenthion	x	x	cis-Nonachlor	x
Malathion	x	x	DCPA (Dacthal)	x
Merphos	x	x	Dicofol	x
Methamidophos (Monitor)	x	x	Dieldrin	x
Methidathion	x	x	Endosulfan Sulfate	x
Methyl Parathion	x	x	Endosulfan-I	x
Mevinphos (Phosdrin)	x	x	Endosulfan-II	x
Phorate	x	x	Endrin	x
Phosmet	x	x	Endrin Aldehyde	x
Tetrachlorvinphos (Stirofos)	x	x	Endrin Ketone	x
Tokuthion	x	x	Heptachlor	x
Trichloronate	x	x	Heptachlor Epoxide	x
			Methoxychlor	x
<b>Pyrethroid Pesticides</b>			Mirex	x
Allethrin	x	x	Oxychlordane	x
Bifenthrin	x	x	Perthane	x
Cyfluthrin	x	x	trans-Nonachlor	x
Cypermethrin	x	x		
Danitol	x	x	<b>Aroclor PCBs</b>	
Deltamethrin	x	x	Aroclor 1016, 1221, 1232, 1242, 1248,	x
Esfenvalerate	x	x	1254, 1260	
Fenvalerate	x	x		
Fluvalinate	x	x	<b>PCB Congeners</b>	
L-Cyhalothrin	x	x	3, 8, 18, 28, 31, 33, 37, 44, 49, 52, 56/60,	x
Permethrin	x	x	66, 70, 74, 77, 81, 87, 95, 97, 99, 101,	
Prallethrin	x	x	105, 110, 114, 118, 119, 123, 126, 128,	
Resmethrin	x	x	138, 141, 149, 151, 153, 156, 157, 158,	
			167, 168+132, 169, 170, 174, 177, 180,	
			183, 187, 189, 194, 195, 200, 201, 203,	
			206, 209	