SWAMP
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Surface Water Ambient Monitoring Program (SWAMP) Toxicity Testing and Toxicity Identification Evaluation

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Toxicity Testing and Toxicity Identification Evaluation

Final Report

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EXECUTIVE SUMMARY

The goals of this study were to conduct analyses of California surface waters for the presence of substances toxic to aquatic life, and to identify the toxicant(s) through toxicity identification evaluation procedures. Water samples were collected by the California Department of Fish and Game/Moss Landing Marine Laboratories, Moss Landing, CA, from sites representing a variety of waterways in Regions 3, 4, 7 and 9 of the Regional Water Quality Control Boards. Toxicity was determined using the zooplankton organism, *Ceriodaphnia dubia*, larval fathead minnows (*Pimephales promelas*) and the green alga *Selenastrum capricornutum* following methods outlined by the US EPA (2002). If the electrical conductivity (EC) of a water sample exceeded 2500 µmhos, the cladoceran *C. dubia* was replaced by the amphipod species *Hyalella azteca* for invertebrate toxicity testing. If the EC exceeded 3500 µmhos, the larval fathead minnow test was replaced by the larval topsmelt (*Atherinops affinis*) test. Toxicity endpoints were reduced survival, production of offspring, and growth. When samples caused 100% mortality within 48 h, dilution series were set up to determine the amount of toxic units. In some cases, toxicity identification evaluation tests (TIEs) were performed to identify the compound(s) causing toxicity when samples caused 50% mortality within 96 h.

Region 3: Among 59 water samples collected from 36 sites in RWQCB Region 3 and tested between January 1, 2006 and March 31, 2007, 28 samples (47.5%) collected from 25 sites (69.4% of sites) were toxic. The majority of toxic samples (44.1%) inhibited growth of green algae. Two water samples collected on January 10, 2006 from Corralitos Creek (305COR001) and on February 27, 2007 from Main Street South (312MSS001) were acutely toxic to fathead minnow larvae. A total of 10 samples (17%) caused toxicity to *C. dubia.* Chronic toxicity to *C. dubia* (reproductive impairment) was seen in four samples collected in January and May 2006; two of them were not toxic to algae or fish, while two of them were also toxic to algae. Six of 13 samples (46.2%) collected in February 2007 were acutely toxic to *C. dubia.* All of these also inhibited algae growth. Toxicity to *C. dubia* and possibly fathead minnow larvae observed in samples collected in February 2007 was mostly due to OP pesticides.

Region 4: No toxicity was detected in the 12 water samples collected and tested in June 2006 from 12 sites in RWQCB Region 4.

Region 7: Of 9 samples collected from 9 sites in Region 7 in May 2006, two (22%) were toxic. Site 723NRBDRY (New River at Boundary) exhibited acute toxicity to *H. azteca*. This site has a history of being toxic due to insecticides (see our 2006 report). The sample from site 719CVSC52 (Coachella Valley Storm Channel, Ave. 52) was acutely toxic to fish and chronically toxic to *C. dubia*. Further testing showed that toxicity was largely due to ammonia.

Region 9: Among the 9 sampling sites in Region 9, 4 sites (44.4%) exhibited toxicity to the algae, and one site (911TTET02, Tecate Creek 2) was toxic on two sampling dates in January and April 2006. Water from this site was also acutely toxic to *C. dubia*. Toxicity at sites 911TTJR05 (Tijuana River 5) and 911TTET02 was not or only partially due to high ammonia levels. Heavy metals were contributing to the toxicity seen on 4/11/06 at site 911TTET02.

BACKGROUND AND APPROACH

The Surface Water Ambient Monitoring Program, or SWAMP, is a statewide monitoring effort designed to assess the conditions of surface waters throughout the state of California. The program is administered by the California State Water Resources Control Board (SWRCB). Responsibility for implementation of monitoring activities resides with the nine California Regional Water Quality Control Boards (RWQCBs) that have jurisdiction over their specific geographical areas of the state. The nine RWQCBs are responsible for protection of water quality within their respective regions of California. RWQCBs apply a variety of monitoring tools to screen surface waters for impairment of aquatic life beneficial uses, including contact recreation (i.e., swimming), fishing, aquatic life and drinking water.

The goals of the study presented here were to conduct analyses of California surface waters for the presence of substances toxic to aquatic life, and to identify the toxicant(s) through toxicity identification evaluation procedures. Samples were collected by the California Department of Fish and Game/Moss Landing Marine Laboratories, Moss Landing, CA, from sites representing a variety of waterways throughout California, and shipped to the UC Davis Aquatic Toxicology Laboratory (UCD ATL), Davis, CA. Toxicity was determined using the zooplankton organism, *Ceriodaphnia dubia*, larval fathead minnows (*Pimephales promelas*) and the green alga *Selenastrum capricornutum* following methods outlined by the US EPA (2002). Toxicity endpoints were reduced survival, production of offspring, and growth. When samples caused 100% mortality within 48 h, dilution series were set up to determine the amount of toxic units. When samples caused 50% mortality within 96 h, toxicity identification evaluation tests (TIEs) were performed to identify the compound(s) causing toxicity. If the EC of a water sample exceeded 2500 µmhos, the cladoceran *C. dubia* was replaced by the amphipod species *Hyalella azteca* for invertebrate toxicity testing. If the EC exceeded 3500 µmhos, the larval fathead minnow test was replaced by the larval topsmelt (*Atherinops affinis*) test and conducted by the UC Davis Granite Canyon Laboratory, Pacific Grove, CA.

This report summarizes and discusses the results of toxicity tests and toxicity identification evaluations performed at the UCD ATL between January 1, 2006 and March 31, 2007 under

agreement number 03-195-250-2 (Master Contract), and contract number 22-1509-3421 (31931) between UCD ATL and the San Jose State University Foundation.

MATERIALS AND METHODS

Sampling Sites

Samples were collected by the State Department of Fish and Game, Moss Landing Marine Laboratories, and shipped to UCD ATL for toxicity tests. Samples were collected as sub-surface grabs (samples collected at 0.1 m below the water surface) in one gallon amber glass jugs to prevent photodegradation. Four gallons of water were collected per site, unless selected for potential Toxicity Identification Evaluations (TIEs), in which case eight gallons were collected. Samples were immediately placed on wet ice in coolers after collection. Sample temperature was maintained between 0-6º C during transport and storage, and toxicity tests were initiated within 48-hours of sample collection. The specifics of the selection of sites are described in more detail in each Region's study plan. Sites sampled for toxicity testing were selected from Region 3 (Central Coast), Region 4 (Los Angeles), Region 7 (Colorado River Basin) and Region 9 (San Diego). The sampling sites for each region are listed below (Tables 1-4).

Table 1: Sampling sites and dates in Region 3 during 2006 and 2007.

Station Code	Station Name	Jan-06	May-07	Feb-07	Latitude	Longitude
304ARA001	Arana Gulch at Harbor High School	1/3/2006	5/30/2006		36.98580	-121.99087
304BRA001	Branciforte Creek at Water Street	1/3/2006	5/30/2006		36.98619	-122.01423
304LOR001	San Lorenzo Estuary at Laurel Street	1/3/2006	5/30/2006		36.96974	-122.02203
304RIV001	San Lorenzo River at Crossing Street	1/3/2006	5/30/2006		36.99083	-122.03096
304SL9001	San Lorenzo River at Highway 9	1/3/2006	5/30/2006		37.08829	-122.08875
304SLB001	San Lorenzo River at Big Trees Road	1/3/2006	5/30/2006		37.02989	-122.05748
304ZAY001	Zayante Creek at Graham Hill Road	1/3/2006	5/30/2006		37.04967	-122.06515
305CAR001	Carnadero Creek at Private property access	1/12/2006	5/17/2006		36.92898	-121.54178
305CHI001	Pajaro River at Chittenden Gap	1/10/2006	5/17/2006		36.90018	-121.59764
305COR001	Salsipuedes Creek - downstream of Corralitos Creek	1/10/2006	5/17/2006		36.91265	-121.74525
305FRA001	Pajaro River at Frazier Lake Road	1/5/2006	5/17/2006		36.96316	-121.49239
305FUF001	Furlong Creek @ Frazier Lake Road	1/5/2006	5/17/2006		36.97808	-121.50912
305HAR001	Harkins Slough at Harkins Slough Road	1/12/2006	5/30/2006		36.90728	-121.80486
305HOL001	Llagas Creek at Holsclaw Road	1/5/2006	5/17/2006		37.03152	-121.54361
305LLA001	Llagas Creek	1/5/2006	5/17/2006		36.97590	-121.51213
305MUR001	Pajaro River @ Murphy's Crossing	1/10/2006	5/17/2006		36.90557	-121.67624
305PAC001	Pacheco Creek	1/12/2006	5/17/2006		36.94385	-121.38488
305PAJ001	Pajaro River at Betabel Road	1/12/2006	5/17/2006		36.91722	-121.54867
305PJP001	Pajaro River at Porter/Main	1/10/2006	5/30/2006		36.90440	-121.75040
305SAN001	San Benito at Y Road	1/10/2006	5/17/2006		36.88504	-121.55324
305SJN001	San Juan Creek @ Anzar	1/10/2006	5/17/2006		36.87566	-121.56129
305UVA001	Uvas Creek at Bloomfield Ave	1/5/2006	5/17/2006		36.96526	-121.53202
305WSA001	Watsonville Slough upstream Harkins Slough	1/12/2006	5/30/2006		36.89002	-121.80218
312ALA001	Alamo Creek at Alamo Creek Road			2/26/2007	35.02667	-120.30370
312BCD001	Blosser Channel d/s of groundwater recharge ponds			2/27/2007	34.98643	-120.45306
312BCU001	Bradley Channel u/s of ponds @ Magellan Drive			2/27/2007	34.97115	-120.41659
312CCC001	Cuyama River d/s Cottonwood Canyon			2/26/2007	35.04076	-119.88570
312CUT001	Cuyama River below Twitchell @ White Rock Lane			2/27/2007	34.90551	-120.30268
312GVT001	Green Valley Trib at Brown Road			2/28/2007		
312MSD001	Main Street Ditch			2/27/2007	34.95428	-120.48167
312MSS001	Main Street South			2/27/2007		
312NIP001	Nipomo Creek			2/26/2007	34.99925	-120.43750
312NIT001	Nipomo Creek @ Tefft Street			2/26/2007	35.03883	-120.48049
312OFL001	Oso Flaco Lake @ culvert			2/28/2007	35.03100	-120.61917
312ORB001	Orcutt Solomon Creek @ Black Road			2/28/2007	34.88386	-120.49406
312ORC001	Orcutt Creek			2/28/2007	34.95633	-120.62870

Table 2: Sampling sites and dates in Region 4 during 2006 and 2007.

Station Code	Station Name	Sample Date	Latitude	Longitude
401VCJVCL	Javon Canyon Lower	6/5/2006	34.33280	-119.40230
401VCJVCU	Javon Canyon Upper	6/5/2006		
401VCMADL	Madranio Lower	6/7/2006	34.34501	-119.41833
401VCMADU	Madranio Upper	6/7/2006		
401VCPJCL	Padre Juan Canyon Lower	6/7/2006	34.31820	-119.36690
401VCPJCU	Padre Juan Canyon Upper	6/7/2006		
401VCSAUL	Los Sauces Lower	6/8/2006	34.34870	-119.42218
401VCSAUU	Los Sauces Upper	6/8/2006		
404VCBSCL	Big Sycamore Lower	6/6/2006	34.07172	-119.01476
404VCBSCU	Big Sycamore Upper	6/6/2006		
404VCLSCL	Little Sycamore Lower	6/6/2006	34.05362	-118.96432
404VCLSCU	Little Sycamore Upper	6/6/2006	34.06479	-118.96310

Table 3: Sampling sites and dates in Region 7 during 2006 and 2007.

Station Code	Station Name	Sample Date	Latitude	Longitude
715CPVLG1	Palo Verde Lagoon (LG1)	5/2/2006	33.43627	-114.71602
715CPVOD2	Palo Verde Outfall Drain (PVOD2)	5/2/2006	33.42260	-114.72615
715CRIDG1	Colorado River at Imperial Dam Grates	5/3/2006	32.88482	-114.46765
719CVSC52	Coachella Valley Stormchannel (Ave 52)	5/2/2006	33.67242	-116.14923
719CVSCOT	Coachella Valley Stormwater Channel Outlet	5/2/2006	33.52444	-116.07778
723ARGRB1	Alamo River Outlet	5/1/2006	33.19920	-115.59710
723ARINTL	Alamo River at International Boundary	5/1/2006	32.67506	-115.37008
723NRBDRY	New River at Boundary	5/1/2006	32.66583	-115.50222
723NROTWM	New River Outlet	5/1/2006	33.10472	-115.66361

Table 4: Sampling sites in Region 9 during 2006 and 2007.

Station Code	Station Name	Jan-06	Apr-06	Latitude	Longitude
908PPAR04	Paradise Creek 4	1/30/2006	4/10/2006	32.66943	-117.10279
908PTEL02	Telegraph Canyon Creek 2	1/30/2006	4/10/2006	32.62853	-117.05751
909SLAW02	Lawson Valley Creek 2		4/11/2006	32.75409	-116.77885
909SSWR03	Sweetwater River 3	1/31/2006	4/11/2006	32.83521	-116.62203
909SSWR08	Sweetwater River 8	1/30/2006	4/10/2006	32.65897	-117.04181
911TCWD10	Cottonwood Creek 10		4/11/2006	32.57300	-116.75753
911TLAP04	La Posta Creek 4	1/31/2006	4/11/2006	32.69997	-116.47959
911TTET02	Tecate Creek 2	1/31/2006	4/11/2006, 4/18/2006	32.56539	-116.75850
911TTJR05	Tijuana River 5		4/10/2006	32.54919	-117.06514

Toxicity Testing

Toxicity testing for fathead minnow larvae (*P. promelas), C. dubia* (a cladoceran, zooplankton species) and the green alga *S. capricornutum* followed the 7 and 4-day static renewal procedures described in "Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms" (US EPA, 2002). Toxicity testing for the amphipod *Hyalella azteca* is based on the procedures outlined in "Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates" (US EPA, 2000). Water quality parameters (EC, pH, dissolved oxygen (DO), ammonia concentration, hardness and alkalinity) were measured on all samples at test initiation; pH and DO were also measured after 24 h at sample renewal. Temperature was monitored continuously. Toxicity tests were initiated within 48-hours of sample collection.

Table 5. Toxicity tests performed on samples from Region 3 during 2006/07[A].

Station Code	*Ceriodaphnia dubia*	*Selenastrum capricornutum*	*Hyalella azteca*	*Pimephales promelas*
304ARA001	x	x		x
304BRA001	x	x		x
304LOR001	x	x		x
304RIV001	x	x		x
304SL9001	x	x		x
304SLB001	x	x		x
304ZAY001	x	x		x
305CAR001	x	x		x
305CHI001	x	x		x
305COR001	x	x		x
305FRA001	x	x		x
305FUF001	x	x		x
305HAR001	x	x		x
305HOL001	x	x		x
305LLA001	x	x		x
305MUR001	x	x		x
305PAC001	x	x		x
305PAJ001	x	x		x
305PJP001	x	x		x
305SAN001		x	x	x
305SJN001		x	x	x
305UVA001	x	x		x
305WSA001	x	x		x
312ALA001	x	x		x
312BCD001	x	x		x
312BCU001	x	x		x
312CCC001		x	x	x
312CUT001	x	x		x
312GVT001	x	x		x
312MSD001	x	x		x
312MSS001	x	x		x
312NIP001	x	x		x
312NIT001	x	x		x
312OFL001	x	x		x
312ORB001	x	x		x
312ORC001	x	x		x

[A:] Region 3 samples were tested with *C. dubia*, *S. capricornutum*, and *P. promelas.* *H. azteca* were used when sample ECs were greater than 2500 µmhos for *C. dubia*.

Table 6. Toxicity tests performed on samples from Region 4 during 2006/07[A].

Station Name	*Ceriodaphnia dubia*	*Selenastrum capricornutum*	*Hyalella azteca*	*Pimephales promelas*
401VCJVCL			x	
401VCJVCU			x	
401VCMADL			x	
401VCMADU			x	
401VCPJCL			x	
401VCPJCU			x	
401VCSAUL				x
401VCSAUU				x
404VCBSCL	x			x
404VCBSCU	x			x
404VCLSCL	x			x
404VCLSCU	x			x

[A]: Region 4 samples were tested with *C. dubia* and *P. promelas*. *H. azteca* were used when sample ECs were greater than 2500 µmhos for *C. dubia*.

Table 7. Toxicity tests performed on samples from Region 7 during 2006/07[A].

Station Name	*Ceriodaphnia dubia*	*Selenastrum capricornutum*	*Hyalella azteca*	*Pimephales promelas*
715CPVLG1	x			x
715CPVOD2	x			x
715CRIDG1	x			x
719CVSC52	x			x
719CVSCOT	x			x
723ARGRB1			x	x
723ARINTL			x	x
723NRBDRY			x	
723NROTWM			x	

[A]: Region 4 samples were tested with *C. dubia* and *P. promelas*. *H. azteca* were used when sample ECs were greater than 2500 µmhos for *C. dubia*.

Table 8. Toxicity tests performed on samples from Region 9 during 2006/07[A].

Station Name	*Ceriodaphnia dubia*	*Selenastrum capricornutum*	*Hyalella azteca*	*Pimephales promelas*
908PPAR04	x	x	x	
908PTEL02		x	x	
909SLAW02	x	x		
909SSWR03	x	x		
909SSWR08	x	x	x	
911TCWD10	x	x		
911TLAP04	x	x		
911TTET02	x	x		
911TTJR05		x	x	

[A]: Region 9 samples were tested with *C. dubia* and *S. capricornutum*. *H. azteca* were used when sample ECs were greater than 2500 µmhos for *C. dubia* due to organism sensitivity to salinity.

Toxicity Testing Protocols

Fathead minnow (Pimephales promelas)

The *P. promelas* chronic tests consist of four replicate 600 ml glass beakers each containing 250 ml of sample and 10 organisms. Tests are initiated with less than 24-hour-old *P. promelas*, which are obtained from Aquatox in Hot Springs, Arkansas. Each replicate is fed freshly hatched *Artemia* nauplii twice daily. Approximately 80% of the test solution is renewed daily, while removing dead fish, *Artemia,* and debris from the test beakers. Deionized water amended to EPA moderately hard (DIEPAMH) is used as the control water for the *P. promelas* test. Tests are conducted in 25 ± 2° C water baths with a 16-hour light: 8-hour dark photoperiod. Mortality is measured daily upon test sample renewal and upon test termination (day 7). At test termination the surviving minnows are anesthetized with MS-222, rinsed with deionized water, dried to constant weight at 103-105° C (approximately 16 hours), and weighed with a Mettler AE 163 balance.

Waterflea (Ceriodaphnia dubia)

The *C. dubia* chronic tests consist of ten replicate 20 ml glass vials each containing one organism. Tests are initiated with less than 24-hour-old *C. dubia*, born within an 8-hour period.

C. dubia are fed a mixture of *S. capricornutum* and YCT (a mixture of yeast, organic alfalfa and trout chow) daily. *C. dubia* are transferred into a new vial of fresh solution daily. Sierra Springs™ water amended to EPA moderately hard (SSEPAMH) water is used as the control water for the *C. dubia* test. Tests are conducted at 25 ± 2° C with a 16-hour light: 8-hour dark photoperiod. Mortality and reproduction (number of neonates) are assessed daily and at test termination (day 7).

Amphipod (Hyalella azteca)

The *H. azteca* 10-day tests consist of five replicate 300 ml glass beakers each containing 100 ml of sample, a one square inch piece of nitex screen (a substrate for the *H. azteca* to cling to), and 10 organisms. Tests are initiated with 7 to 14 day old *H. azteca*, which are obtained from Aquatic Research Organisms in Hampton, New Hampshire. Animals in each replicate are fed 100 µl YCT on days 0, 2 and 4, after water renewal on day 5, and on day 6. Approximately 75% of the test solution is renewed on day 5. Deionized water reconstituted to EPA moderately hard and adjusted to 3000 µmhos with seawater (3000µmhos DIEPAMHR) is used as the control water. The DIEPAMHR is adjusted to a conductivity of 3000 µmhos, because only sample waters that exceed 2500µmhos are tested with *H. azteca.* Tests are conducted in a 23 ± 2° C chamber with a 16-hour light: 8-hour dark photoperiod. Mortality is measured daily and at test termination (day 10).

Green algae (Selenastrum capricornutum)

The *S. capricornutum* 96-hour chronic tests consist of four replicate 200 ml glass flasks with 100 ml of sample and 1 ml of 1.0×10^6 cell/ml *S. capricornutum*. Glass distilled water is the control for the *S. capricornutum* test. Test chambers are incubated in a temperature-controlled environmental chamber maintained at 25 ± 2º C under continuous cool white fluorescent light. Test chambers are kept in a mechanical shaker in constant orbital motion at 100 cycles/minute; flasks are randomized twice daily. Growth is measured at test termination (day 4).

UCD ATL often receives sample waters from the Regional Boards that have electrical conductivities (EC) that exceed 1000 µmhos. High ECs could potentially cause a statistically significant decrease in algae cell count, suggesting that a toxicant may be present in the sample,

when in fact the cell count reduction is due to EC. An algae study was conducted to determine how elevated ECs would affect cell count, so a differentiation could be made between toxicity and elevated EC effects. The EC of glass distilled (GD) water was increased to 1000, 2000, and 3000 µmhos using either sodium chloride (NaCl) or seawater. The GD water adjusted to 2000 and 3000 µmhos, whether it was adjusted with seawater or NaCl, exhibited a statistically significant reduction in cell count as compared to the GD water control. The cell count of the 3000 µmhos GD water adjusted with seawater was significantly lower than the 3000 µmhos GD water adjusted with NaCl. These results prompted the UCD ATL to begin including high EC controls if there were sample waters that exceeded 1500 µmhos. The high EC control water consists of GD water adjusted to 1500 µmhos using filtered Pacific Ocean seawater. Seawater is used to adjust the GD water because its composition better represents that of high EC sample waters. However, it is understood that ionic ratios in various surface waters can differ from those in seawater. UCD ATL understands that ideal controls for these tests would mimic the EC and ionic composition of the surface waters being tested. However, funding precludes this level of detail in testing ambient samples. Samples with elevated ECs are indicated as such in each Region's *S. capricornutum* section of the Appendix.

Dilution Series

Dilution series toxicity tests (five 50% dilutions) were conducted on samples that caused 100% mortality in the test organisms within 48 hours of test initiation. Dilution series tests are performed to estimate the magnitude of toxicity in a toxic sample. Results of these tests are used to estimate the number of toxic units (TUs) in a sample. A TU is defined as the concentration of a specific chemical present in a sample divided by the 96-hour LC50 concentration for the species of interest. An LC50 is defined as the concentration of a chemical that causes 50% mortality in 96 hours. Toxic units can be added when multiple toxicants are present (assuming that the individual toxic compounds act additively) to equal the total number of toxic units. Toxic units contributed by individual toxicants can be compared to toxic units, which are determined by percent dilutions of the ambient water sample at 100, 50, 25, 12.5, and 6.25. Dilution series consist of five dilutions, where samples are diluted with control water. Such dilution series toxicity tests precede a Phase I TIE.

Toxicity Identification Evaluations (TIEs)

Phase I TIEs were conducted on toxic samples at the Contract Manager's request to identify the class(es) of contaminant(s) causing the observed toxicity. Phase I TIEs involved procedures to either remove or inactivate specific classes of chemicals (US EPA, 1991). After manipulation, the toxicity of a sample was tested and compared to the original water sample. Improved organism performance following TIE manipulation was defined as the absence or a delay of mortality by greater than or equal to 24 hours. Phase I TIEs manipulations included air-stripping, Disodium Ethylenediamine Tetraacetate (EDTA) addition, Sodium Thiosulfate (STS) addition, Piperonyl Butoxide (PBO) addition, and solid phase extraction (C8-SPE). Various Phase I TIE manipulations and how the results can be interpreted are outlined in Table 9 below.

Table 9. Summary of TIE manipulations and interpretations

TIE Manipulations	Interpretations
Species-specific Control	Unmanipulated Control
Control (HA) @ 'X' mg/L $CaCO_3$	Control adjusted to the hardness of the ambient sample, to determine if hardness has any effect on toxicity.
Control (HA) + MeOH @ 0.5%	Solvent Control
Control (HA) + eluate addback @ 3x	Concentrated eluate from the ambient sample extracted from the C8 column added back to a control. If toxicant in question is a non-polar organic compound, this will exhibit accelerated mortality compared to the ambient sample. If this treatment exhibits no mortality, toxicant is not a non-polar organic
Control (HA) + 'X-1' mg/L EDTA	EDTA and STS Method controls to determine if there is any artifactual toxicity due to the manipulations themselves. Concentrations of EDTA and STS are determined by the hardness of the ambient sample.
Control (HA) + 'X-2' mg/L EDTA	
Control (HA) + 'X-3' mg/L STS	
Control (HA) + 'X-1' mg/L STS	
Control (HA) + 'X-2' mg/L STS	
Control (HA) + 'X-3' mg/L STS	
Control (HA) + 25 ppb PBO	PBO Control
Control (HA) air stripped	Air-stripping Method Control to determine any artifactual toxicity resulting from air stripping manipulations
Control C8 blank	Control water is passed through an C8 column and serves as one of the method controls (blank) to ascertain that no toxicity is introduced from the column alone.
Ambient Sample	Unmanipulated ambient sample is retested to determine if the toxicant is still present and may serve as a statistical comparison for various manipulations.
Ambient Sample + 'X-1' mg/L EDTA	If the toxicant is a metal(s), the unmanipulated ambient sample exhibits high mortality while the sample amended with EDTA or STS results in reduced or no mortality.
Ambient Sample + 'X-2' mg/L EDTA	
Ambient Sample + 'X-3' mg/L EDTA	
Ambient Sample + 'X-1' mg/L STS	
Ambient Sample + 'X-2' mg/L STS	
Ambient Sample + 'X-3' mg/L STS	
Ambient Sample + 25 ppb PBO	If the toxicant is a metabolically activated chemical such as an OP insecticide, the unmanipulated ambient sample will cause high mortality while the ambient sample amended with PBO results in reduced or no mortality. However, if the toxicant is a carbamate or pyrethroid, both the manipulated and unmanipulated samples will exhibit high mortality.
Ambient Sample air stripped	Aerating the ambient sample prior to analysis can remove toxicity due to a surfactant or a volatile compound.
Ambient Sample C8 Rinsate	If the toxicant is a non-polar organic chemical, the ambient sample and control water amended with column eluate will exhibit mortality while the sample “rinsate” will exhibit reduced or no mortality

Heavy metals can be toxic to aquatic species if concentrations exceed threshold levels. EDTA and STS bind to various metals, making them unavailable to biota. Three concentrations of each EDTA and STS are added to toxic samples and tested along with the appropriate controls. If the toxicant is a metal(s), the unmanipulated sample exhibits high mortality while the sample amended with EDTA or STS results in reduced or no mortality.

PBO decreases toxicity by retarding or preventing formation of the toxicologically active forms of diazinon and chlorpyrifos (Bailey *et al*., 1996). It has no effect on carbofuran, a carbamate insecticide, but potentiates the toxicity of pyrethroid insecticides. PBO is added to the toxic samples for a final concentration of 0.1 ppm in *C. dubia* and *H. azteca* TIEs. The unmanipulated sample and the sample amended with PBO are tested along with the appropriate controls in a toxicity test. If the toxicant is a metabolically activated OP insecticide, the unmanipulated test sample will cause high mortality while the test sample amended with PBO results in reduced or no mortality. However, if the toxicant is a carbamate or pyrethroid, both the manipulated and unmanipulated samples will exhibit high mortality.

SPE columns primarily remove non-polar organic chemicals from water samples. A toxic sample is passed through an SPE column. Control water also is passed through an SPE column and serves as one of the method controls (blank) to ascertain that no toxicity is introduced from the column alone. The column is then eluted with methanol and the eluate added to control water and tested along with the appropriate method control, the untreated ambient sample and the column "rinsate" (ambient sample passed through SPE column). If the toxicant is a non-polar organic chemical, the ambient sample and control water amended with column eluate will exhibit mortality while the sample "rinsate" will exhibit reduced or no mortality.

Air stripping reduces or removes toxicity caused by chemicals such as surfactants, chlorine and/or ammonia from waters. Toxic samples are air stripped and tested along with the appropriate control. If the toxicant is a volatile compound, the ambient sample exhibits high mortality while the air-stripped sample results in reduced or no mortality. Recently, work performed at UCD ATL documented that air-stripping of a water sample spiked with a non-

volatile insecticide reduced *C. dubia* mortality. Results from this TIE manipulation should therefore be interpreted with caution.

Toxic samples are amended with carboxlyesterase to reduce or remove pyrethroid-associated toxicity (Wheelock *et al*. 2005). This method is still in its experimental phase, and results need to be interpreted with caution. Previous studies conducted at the University of California, Davis Aquatic Toxicology Laboratory have demonstrated the effectiveness of carboxlyesterase to reduce or remove pyrethroid-associated toxicity in water column bioassays. Stock solutions are made the day of test initiation, using liquid esterase with an initial activity level of 0.0025 units/mL, and added to the appropriate control water for the species used. Deionized water amended to US EPA moderately hard specifications (DIEPAMHR) was used for the *H. azteca*. Esterase was added at a concentration of 500 units/ml. This assay alone is not sufficiently specific to identify pyrethroids as the dominant toxicants, because hydrophobic chemicals can be removed from the water sample by unspecific binding to the esterase molecules. However, laboratory experiments indicate that toxicity reduction due to unspecific binding of contaminants to proteins is relatively small. In addition, in conjunction with an increase in toxicity after PBO addition, the reduction of toxicity after esterase addition provides strong indication for the presence of toxic concentrations of pyrethroids. An additional protein control (bovine serum albumin, BSA) can be used to account for unspecific removal of toxic chemicals.

When ammonia toxicity was suspected based on a high ammonia measurement, the pH of the water sample was adjusted to 7.3 and 6.3. Ammonia levels that exceed 5 mg/L are toxic to aquatic organisms. At lower pH levels unionized ammonia (NH_3) is converted to ionic ammonium (NH_4^+), which is less toxic to aquatic organisms. Reduction in toxicity at low pH confirms that ammonia was responsible for the observed toxicity.

No Phase II TIEs were conducted during this reporting period.

Chemical Analyses

Enzyme-Linked Immunosorbent Assays (ELISA) were used to determine diazinon and chlorpyrifos concentrations. These measurements were taken on samples that had been homogenized prior to analysis. QA/QC for ELISA analysis consisted of blanks, spikes and duplicates. Results are outlined in Tables A32-A36 in the Region 3 Water Quality section of the Appendix.

Water Chemistry

Prior to *C. dubia, S. capricornutum, P. promelas,* and *H. azteca* test initiation, temperature, dissolved oxygen (DO), pH, electrical conductivity (EC), alkalinity, hardness, and ammonia were measured. The *S. capricornutum* test had pH measured daily. In the *C. dubia* and *P. promelas* tests, DO was measured in the daily renewal water, and DO and pH were measured in the 24 hour old water. In the *H. azteca* test, DO was measured in the renewal water on day 5, and DO and pH were measured in the 5-day old water. Temperature was monitored continuously throughout all toxicity tests.

Quality Assurance/Quality Control (QA/QC)

All UCD ATL procedures followed a stringent QA/QC plan approved by the Contract Manager and consistent with the US EPA QA guidelines and the QAMP established for the SWAMP program. Toxicity tests were initiated within 48 h of sample collection. To assess repeatability, laboratory control duplicates and field duplicates were tested. To determine whether test species were responding typically, positive reference toxicant tests were conducted concurrently with each batch of samples, or monthly (if samples arrived after the 15th of the month) to ascertain organism health, sensitivity and laboratory performance. All tests were evaluated by the UCD ATL Quality Assurance Officer and met specified US EPA criteria. All QA/QC data are presented by region in the Appendix.

Data Analysis and Reporting

Each sample is characterized by descriptive statistics indicating the mean response and variation among replicates. Statistical comparisons consist of t-tests that compare the response of test organisms in sample water to the response in laboratory control water.

Toxicity is defined as a statistically significant mortality difference ($p<0.05$) in an ambient sample compared to a laboratory control. Specifically, acute toxicity in the *C. dubia* and larval *P. promelas* toxicity assays is defined as statistically significant mortality within 96 hours in a test sample compared to the laboratory control. Toxicity in the *S. capricornutum* toxicity assay is defined as statistically significant reduction in cell growth when compared to a laboratory control at test termination. When toxicity is detected, the SWRCB CM and RWQCB Contacts will be notified within 24 hours.

All toxicity data is analyzed using the Microsoft Excel ToxConverter macro written by the SWAMP data management team. This macro compares control and experimental treatments by performing one-tailed t-tests that do not assume homogeneity of variance. Prior to the October 2005 Region 7 sampling event, the alpha level was Bonferroni-corrected to maintain the stated probability of Type I error on the level of the entire toxicity batch. Analysis of the October 2005 Region 7 data did not include Bonferroni correction. Bonferroni correction was discontinued in order to standardize the analysis of all data entered into the SWAMP database, according to the decision made during the SWAMP QA conference call on 12/7/05.

All data produced during this period were quality assurance checked and entered into SWAMP Excel spreadsheets. Spreadsheet entries were double checked for accuracy and have been submitted to Moss Landing Marine Laboratories to be entered into the SWAMP database.

A UCD ATL representative attended the monthly SWAMP meetings and communicated monthly with the Contract Manager and the Regional Board representatives. Data and information regarding the toxicity tests conducted during the project were reported to the Contract Manager and Regional Board representatives within the time frame stipulated in the contract.

RESULTS

January 1 – June 30, 2006

Region 3

Selenastrum capricornutum

Results of the *S. capricornutum* tests conducted on samples collected between January 3, 2006 and May 30, 2006 are summarized in Tables A1-6 of the Data Appendix. Sixteen samples exhibited statistically significant reduction ($p<0.05$) in cell count as compared to the control: 304ARA001, 304BRA001, 304RIV001 (samples collected January 3, 2006); 305SJN001, 305SAN001, 305CHI001, 305MUR001, 305COR001 (samples collected January 10, 2006); 305HAR001, 305FDQ006, 305WSA001, (samples collected January 12, 2006); 305FUF001, 305SAN001, 305SJN001 (samples collected May 17, 2006); 305HAR001, and 305FQA001 (samples collected May 30, 2006).

Two of the sixteen Region 3 samples that were toxic to *S. capricornutum* had ECs greater than 1500 µmhos: 305SJN001 and 305SAN001, both collected January 10, 2006. A high EC control, matched to the ECs of the ambient samples, was included in the initial screening test. The high EC control exhibited a statistically significant reduction ($p<0.05$) in cell growth as compared to the unaltered control. These results suggest that the toxicity exhibited by 305SJN001 and 305SAN001 may be due to high EC rather than potential contaminant(s) present in the samples.

Ceriodaphnia dubia

The results of the *C. dubia* chronic toxicity tests conducted on samples collected between January 3, 2006 and May 30, 2006 are summarized in Tables A10-13 of the Data Appendix. Four samples exhibited a statistically significant reduction ($p<0.05$) in reproduction as compared to the control: 304ZAY001 (collected January 3, 2006), 305CAR001 (collected January 12, 2006), 305FUF001 (collected May 17, 2006), and 305SJN001 (collected May 17, 2006). There were no statistically significant reductions in survival in any of the samples.

Pimephales promelas

Results of the *P. promelas* chronic toxicity tests conducted on samples collected between January 3, 2006 and May 30, 2006 are summarized in Tables A17-25 of the Data Appendix. Sample 305COR001 (collected January 10, 2006), caused statistically significant ($p<0.05$) reductions in biomass and survival as compared to the control. This sample did not exhibit toxicity (although a trend to reduced survival was observed) in the initial screening test, but a follow-up experiment aimed at reducing variability due to pathogen-related mortality did elicit a toxic result. Due to shortage of sample water, there was no follow-up testing.

Hyalella azteca

Results of the *H. azteca* chronic toxicity test conducted on samples collected on January 10, 2006 are summarized in Table A29 of the Data Appendix. There were no statistically significant reductions in survival in the test.

Toxicity Identification Evaluations

There were no TIEs conducted for this Region in 2006.

ELISA Analysis for Diazinon and Chlorpyrifos

All samples collected for Region 3 were analyzed for Chlorpyrifos and Diazinon using enzyme-linked immunosorbent assays (ELISAs). The results of the ELISA analyses are shown in Tables A32-35 in the Water Chemistry section of the Data Appendix.

Water Chemistry

Water chemistry measurements taken in 2006 are summarized in Tables A37-42 in the Water Chemistry Data Appendix. Water chemistry measurements fell within the tolerance ranges for *S. capricornutum, C. dubia, P. promelas* and *H. azteca*.

Region 4

Ceriodaphnia dubia

Results of the *C. dubia* chronic toxicity tests conducted on samples collected between June 5 and June 8, 2006 are summarized in Table A54 of the Data Appendix. There were no statistically significant reductions in survival or reproduction in the tests.

Pimephales promelas

Results of the *P. promelas* chronic toxicity tests conducted on samples collected between June 5 and June 8, 2006 are summarized in Tables A55-56 of the Data Appendix. There were no statistically significant reductions in survival or biomass in the tests.

Hyalella azteca

Results of the *H. azteca* chronic toxicity test conducted on samples collected between June 5 and June 8, 2006 are summarized in Tables A57-58 of the Data Appendix. There were no statistically significant reductions in survival in the test.

Water Chemistry

Water chemistry is summarized in Tables A59-62 in the Data Appendix. Water chemistry measurements fell within the tolerance ranges for *C. dubia*, *P. promelas* and *H. azteca*.

Region 7

Ceriodaphnia dubia

Results of the *C. dubia* chronic toxicity tests conducted on samples collected between May 1 and 2, 2006 are summarized in Tables A66-67 of the Data Appendix. Sample 719CVSC52, collected on May 1, 2006, exhibited a statistically significant reduction ($p<0.05$) in reproduction as compared to the control. There were no statistically significant reductions in survival in the tests.

Pimephales promelas

Results of the *P. promelas* chronic toxicity tests conducted on samples collected on May 1 and 2, 2006 are summarized in Tables A68-70 of the Data Appendix. Sample 719CVSC52, collected

on May 1, 2006, exhibited statistically significant reductions ($p<0.05$) in survival and biomass as compared to the control.

An acute graduated pH shift test was initiated with sample 719CVSC52, in addition to its use in the initial screening test because the sample contained 12 mg/L of ammonia. In the graduated pH shift test, 719CVSC52 was initiated in one treatment with its pH of 7.74 unaltered. A second treatment was initiated with the sample's pH lowered to 6.74, as well as a third treatment with a lowered pH of 5.74. The unaltered 719CVSC52 exhibited 100% mortality in the test. 719CVSC52 with its pH lowered to 6.74 exhibited 45% mortality in the test. 719CVSC52 with its pH lowered to 5.74 exhibited 5% mortality in the test. These results suggest that the toxicity exhibited in the initial screening test may be due to ammonia, as pH-adjusting the sample significantly reduced toxicity.

Hyalella azteca

Results of the *H. azteca* chronic toxicity tests conducted on samples collected on May 1 and 2, 2006 are summarized in Tables A71-72 of the Data Appendix. Sample 723NRBDRY collected May 2, 2006 exhibited a statistically significant reduction in survival as compared to the control.

Toxicity Identification Evaluations

One Phase I TIE was conducted on an acutely toxic sample.

Sample 723NRBDRY, collected May 2, 2006, caused 100% mortality within 48 hours to *H. azteca* in the initial screening test. A dilution series test was initiated. The *H. azteca* exhibited 100% mortality within 72 hours in the 100 and 50% dilutions, 74% mortality within 96 hours in the 25% dilution, and 34% mortality within 96 hours in the 12.5% dilution. These results indicate that there were approximately 6 Toxic Units (TU) present in the sample. The results of the dilution series is shown in Table A73 of the Data Appendix.

Phase I TIE results (Table A74) indicate that the toxicity exhibited in the initial screening test may have been due to surfactants and/or volatile compounds, as toxicity was removed in the TIE by aerating the sample. There were several problems encountered with the methanol

manipulations in the Phase I TIE; follow-up studies indicated that the methanol source was contaminated. However, the Phase I TIE demonstrated that a non-polar organic compound may have contributed to the toxicity, as the eluate add-back manipulation exhibited accelerated mortality compared to the C8 column rinsate.

Water Chemistry

Water chemistry is summarized in Tables A76-81 in the Data Appendix. Water chemistry fell within the tolerance ranges of *C. dubia*, *P. promelas* and *H. azteca*.

Region 9

Selenastrum capricornutum

Results of the *S. capricornutum* chronic toxicity tests conducted on samples collected between January 13, 2006 and April 18, 2006 are summarized in Tables A83-84 of the Data Appendix. Four sites exhibited statistically significant reduction ($p<0.05$) in cell count as compared to the control: 911TLAP04, 909SWR08, 911TTET02 (samples collected January 30 and 31, and April 10 and 11, 2006); 911TTJR05. These samples had ECs greater than 2000 μmhos. A high EC control was included in the initial screening tests. Toxicity in these samples may be due to high EC, since their EC was similar to the EC of the high EC controls, which also exhibited reduced cell growth.

Ceriodaphnia dubia

The results of the *C. dubia* chronic toxicity test conducted on samples collected between January 13, 2006 and April 18, 2006 are summarized in Tables A85 and A87 of the Data Appendix. One sample exhibited multiple statistically significant reductions in survival as compared to the control: 911TTET02, collected January 31, 2006, and again on April 10, 2006.

An acute graduated pH shift test was initiated with sample 911TTET02, collection date January 31, 2006, in addition to its use in the initial screening test because the sample contained 18 mg/L of ammonia. Ammonia levels that exceed 5 mg/L are toxic to test organisms. At lower pH levels, ammonia is converted to ammonium, which is less toxic to aquatic organisms. In the graduated pH shift test, 911TTET02 was initiated in one treatment with its pH of 8.24 unaltered.

A second treatment was initiated with the sample's pH lowered to 7.24, as well as a third treatment with a lowered pH of 6.24. All three of the ambient treatments exhibited 100% mortality in the test (results are shown in Table A86 in the *C. dubia* Data Appendix). Upon test termination, the ammonia concentration was measured in the two pH-manipulated ambient treatments, which remained high at 15 mg/L and 16 mg/L, respectively. These results suggest that the toxicity exhibited in the initial screening test may partly be due to ammonia, as pH-adjusting the samples reduced the ammonia levels from 18 mg/L to 15 mg/L. However, adjusting the pH did not completely remove toxicity from the sample, which suggests that another unknown contaminant may have contributed to the toxicity.

A second acute graduated pH shift test was initiated with sample 911TTET02 (Appendix, Table A88), collection date April 10, 2006, in addition to its use in the initial screening test because the sample contained 16 mg/L of ammonia. A dilution series indicated the presence of 1-2 toxic units (Appendix, Table A89). In the graduated pH shift test, 911TTET02 was initiated in one treatment with its pH of 8.34 unaltered. A second treatment was initiated with the samples pH lowered to 7.34, as well as a third treatment with a lowered pH of 6.34. The unaltered 911TTET02 exhibited 100% mortality in the test. 911TTET02 with its pH lowered to 7.34 exhibited 50% mortality in the test. 911TTET02 with its pH lowered to 6.34 exhibited 100% survival in the test. These results suggest that the toxicity exhibited in the initial screening test may be due to ammonia.

Hyalella azteca

Results of the *H. azteca* chronic toxicity test conducted on samples collected between January 13 and April 18, 2006 are summarized in Tables A890-92 of the Data Appendix. Sample 911TTJR05, collected April 10, 2006, exhibited a statistically significant reduction in survival as compared to the control. Prior to test initiation, 911TTJR05 had a dissolved oxygen (DO) concentration of 1.7 mg/L. DO concentrations that are less than 4 mg/L are considered toxic to aquatic organisms. An additional treatment of 911TTJR05 was included in the initial screening test with constant aeration applied in order to mitigate the effects of the low DO concentration. Slow, constant aeration set at 100 bubbles/min via an air bar was administered to the treatment (Table A91).

In addition, an acute graduated pH shift test was initiated with sample 911TTJR05, because the sample contained 24 mg/L of ammonia. In the graduated pH shift test, 911TTJR05 was initiated in one treatment with its pH of 7.61 unaltered. A second treatment was initiated with the sample's pH lowered to 6.61, as well as a third treatment with a lowered pH of 5.61. All three ambient treatments exhibited 100% mortality in the test (results summarized in Table A93 in the Data Appendix). These results suggest that the combination of high ammonia and low DO concentrations, as well as some other factor, may have contributed to the toxicity exhibited in the initial screening test.

Toxicity Identification Evaluations

One Phase I TIE was conducted on an acutely toxic sample. The results of the dilution series and TIEs are shown in Tables A94-96 of the Data Appendix.

Sample 911TTET02, collected April 10, 2006, caused 100% mortality within 24 hours to *C. dubia.* A dilution series test was initiated. The *C. dubia* exhibited 100% mortality within 48 hours in the undiluted sample, and 100% survival in all dilutions. These results indicate that there were between one and two TUs present in the sample.

Phase I TIE results indicate that the toxicity exhibited in the initial screening test may have been in part, due to a cationic metal(s), as mortality was significantly delayed, relative to the ambient sample, in the treatments amended with sodium thiosulfate and EDTA. Moreover, there was an absence of toxicity in the C8 column eluate add-back manipulation, and toxicity was still present in the C8 column rinsate as well as the aerated ambient treatment, which implicates that the toxicity was not due to non-polar organic compounds, volatile chemicals and/or surfactants.

Water Chemistry

Water chemistry is summarized in Tables A97-107 in the Data Appendix. Water chemistry measurements fell within the tolerance ranges of *S. capricornutum*, *C. dubia* and *H. azteca*.

July 1, 2006 – March 31, 2007

Region 3

Selenastrum capricornutum

Results of the *S. capricornutum* tests conducted on samples collected February 26/27, 2007 are summarized in Tables A7 – 9 of the Data Appendix. The following samples caused a significant reduction in cell count due to other stressors: 312ALA001, 312NIT001, 312BCD001, 312MSD001, 312MSS001, 312OFL001, 312ORC001, 312GVT001 and 312ORB001.

High salinity was the likely cause for the statistically significant reduction ($p<0.05$) in cell count in samples 312CCC001 and 312NIP001. These samples had ECs greater than 1500 µmhos, which is the tolerance limit for *S. capricornutum*. A high EC control, matched to the ECs of the ambient samples (approx. 2000 µmhos), was included in the test. The high EC control exhibited a statistically significant reduction ($p<0.05$) in cell growth as compared to the unaltered control, suggesting that the toxicity measured was likely due to high EC rather than potential contaminant(s) present in the samples.

Ceriodaphnia dubia

The results of the *C. dubia* chronic toxicity tests conducted on samples collected February 26/27, 2007 are summarized in Tables A14-16 of the Data Appendix. Six samples caused a statistically significant reduction (100%) in survival as compared to the control: 312BCD001, 312MSD001, 312MSS001, 312ORC001, 312GVT001 and 312ORB001. Mortality was likely due to organophosphate insecticides (see ELISA results below).

Pimephales promelas

Results of the *P. promelas* chronic toxicity tests conducted on samples collected February 26/27, 2007 are summarized in Tables A26-28 of the Data Appendix. Sample 312MSS001 caused 100% mortality. A partial TIE was conducted to identify the causes of the toxicity (see below).

Hyalella azteca

Sample 312CCC001 was tested with *H. azteca* due to high conductivity. Results of the chronic toxicity test are summarized in Table A30 of the Data Appendix. There was no statistically significant reduction in *H. azteca* survival.

Toxicity Identification Evaluations

Sample 312MSS001 collected on 2/27/2007 caused 100% mortality in fathead minnow larvae. A pH shift TIE was conducted to determine if ammonia was the possible cause of toxicity (Appendix, Table A31). A shift to pH 6.0 reduced short-term (24 h) toxicity of this sample, indicating that ammonia was, in part, causing the toxicity. However, other toxicants, such as organophosphate insecticides, were likely contributing to the toxicity, as indicated by the presence of diazinon and chlorpyrifos in ELISA analyses (see below).

ELISA Analysis for Diazinon and Chlorpyrifos

All samples collected for Region 3 were analyzed for Chlorpyrifos and Diazinon using enzyme-linked immunosorbent assays (ELISAs). The results of the ELISA analyses are shown in Table A36 in the Data Appendix. Samples 312BCD001, 312MSD001, 312MSS001, 312ORC001, 312GVT001 and 312ORB001 contained toxic concentrations of diazinon and chlorpyrifos. In some instances, toxicity was not observed in the initial screening tests conducted with samples in which ELISA results indicated toxic concentrations of chlorpyrifos. UCD ATL did not send any samples out for chemical analyses to verify the ELISA results. Therefore, the organisms' insensitivity cannot be explained.

Quality Assurance/Quality Control

UCD ATL tests approximately 10% of all samples for QA/QC determinations. Over the course of this project, 14 samples were selected for QA (approximately 13%). These samples included the following: 6 field duplicates, 4 bottle blanks and 4 travel blanks. A field duplicate is a second sample that is collected directly after the primary sample and treated in the same manner. It is used to evaluate precision. A bottle blank is prepared in the laboratory and is an analyte-free water sample (e.g., laboratory control water) that is transferred to a clean sample container. It is used to evaluate potential contamination due to the sample container or laboratory cleaning

methods. A travel blank is an analyte-free water sample that is transferred into a clean sample container and is prepared in the laboratory, brought out into the field, and treated like any other water sample (but never opened up) throughout the course of the trip. It is used to evaluate potential incidental contamination that can occur during field sampling and sample processing. SWAMP requirements dictate that field duplicates be collected at a rate of 5% over the course of a project. UCD ATL and the DFG sampling teams met that requirement with 6 field duplicates. No other applicable QC samples are required by SWAMP for toxicity testing. Bottle and travel blanks were included in this project at the discretion of the QA Officer.

UCD ATL supplies the DFG sampling teams with pre-cleaned one-gallon amber bottles directly from the manufacturer; thus bottle blanks would not usually be a component in QC determinations. However, laboratory-cleaned amber bottles were used for the travel blank samples that were conducted with the February 2007 sample dates. Therefore, it was considered appropriate to also include bottle blanks with the same batch of sampling containers used for the travel blanks. The overall performance of the QA/QC samples is outlined in Table 9 below. A more detailed description of QA/QC sample performance for each Region can be found in the Data Appendix.

Precision: Precision is the degree to which the primary sample agrees with its duplicate. Field duplicate samples are in agreement when they are both either statistically similar or statistically different from the laboratory control. Precision can be determined from bottle and travel blanks as well, when the blank in question is statistically similar to the laboratory control. The results are considered equivalent. Precision can be measured by calculating the Relative Percent Difference (RPD) between sample measurements in field duplicates. The RPD between a sample and its duplicate can be calculated by using the following equation:

$$RPD = \left(\frac{\left[2 * \left| Dup1 - Dup2 \right| \right]}{\left[Dup1 + Dup2 \right]} \right) * 100$$

For this project, RPDs were calculated the aforementioned equation on water chemistry measurements such as DO, pH, EC, hardness, alkalinity, and ammonia. Both the individual and average RPDs between duplicates are listed in detail in the Data Appendix (Region 3: Tables

A46-53; Region 4: Tables A66-65; Region 7: Table A82; Region 9: Tables A108-110). The frequency of field duplicates sharing equivalent results is outlined in Table 10.

Table 10. Frequency of QA/QC samples sharing equivalent results – entire project

Species Endpoint	Field Duplicates		Bottle Blanks		Travel Blanks	
	Sample Size	% Agreement	Sample Size	% Agreement	Sample Size	% Agreement
C. dubia **survival**	6	100	1	100	1	100
C. dubia **reproduction**	6	100	1	100	1	100
P. promelas **survival**	5	100	1	100	2	100
P. promelas **biomass**	5	100	1	100	2	100
H. azteca **survival**	0	NA	1	100	0	NA
Algae **growth**	5	100	1	100	1	100

ELISA Analysis: ELISAs were conducted on samples collected from Region 3 to detect the presence of chlorpyrifos and diazinon. Detailed ELISA results can be found in the Region 3 water quality section of the appendix. With regards to QC requirements, several issues came about during the ELISA analysis process over the course of this project.

For samples collected 1/4/06 and 1/5/06, E-standards were not included in the ELISA process for both analytes. Additionally, the matrix spike fell short of meeting the SWAMP matrix spike results requirement of 200-300 ng/L for both analytes. These samples were not re-run in any additional analyses. For samples collected 1/13/06, E-standards were not included in the ELISA process, and the matrix spike did not meet the SWAMP range of 200-300 ng/L for both analytes. These samples were not re-run in any additional analyses. For samples collected 5/17/06 and 5/18/06, E-standards were not included in the ELISA process for both analytes. Sample 305LLA001 exceeded the SWAMP %CV requirement for diazinon, and sample 305SAN001 exceeded the SWAMP %CV requirement for both diazinon and chlorpyrifos. Matrix spike

results for both diazinon and chlorpyrifos exceeded the SWAMP requirement of 200-300 ng/L. This was likely due to technician error rather than the presence of an analyte already in the sample, for the sample chosen for the matrix spike was a non-detect. These samples were not re-run in any additional analyses. For samples collected 5/31/06, E-standards were not included in the ELISA process for both analytes. The matrix spike for chlorpyrifos did not meet the SWAMP minimum requirement of 200-300 ng/L, and the matrix spike for diazinon exceeded the maximum requirement of 300 ng/L. Exceeding of the maximum matrix spike requirement for diazinon is likely due to technician error rather than the presence of an analyte already in the sample, for the sample chosen for the matrix spike was a non-detect.

For samples collected 2/26/07 – 2/28/07, an E-standard was included in the analyses, but failed to meet the SWAMP minimum requirement of 200-300 ng/L. Field duplicate RPDs exceeded SWAMP requirements for both analytes. Matrix spike results exceeded the SWAMP requirement for both analytes. However, this was due to analyte(s) already present in the sample(s) chosen for the matrix spike. The sample chosen (312BCD001) for the matrix spike had a chlorpyrifos concentration of 410 ng/L, which with the addition of the 250 ng/L used for the spike, brings the total possible amount of chlorpyrifos detected to approximately 660 ng/L. Results of the chlorpyrifos matrix spike show a detection of 711 ng/L. 312BCD001 chosen for the matrix spike exceeded the kit's upper detection limit for diazinon, which had to be diluted and re-run. The diluted sample had a diazinon concentration of 977 ng/L. The addition of the 250 ng/L used for the spike brings the total possible amount of diazinon detected to approximately 1227 ng/L. Results of the diazinon matrix spike indicate a detection of 1219 ng/L. An additional sample (312GVT001) was chosen for a second matrix spike and was re-run in an additional analysis but also exceeded the SWAMP matrix spike requirement for both analytes. 312GVT001 chosen for the secondary matrix spike also exceeded the kit's upper detection limit for chlorpyrifos and diazinon, and was diluted and re-run.

UCD ATL experienced the following limitations while conducting ELISAs:
E-standards: External standards are used as a reference to determine kit precision, accuracy and sensitivity over time. As E-standards were not included in any of the ELISA analyses conducted in 2006, those endpoints cannot readily be determined.

Matrix spikes: Matrix spikes are used to evaluate the effect a particular sample matrix has on the accuracy of a measurement. In the ELISAs conducted in 2006, detection results were generally lower than expected. We assume that this was due to technician error, but it is also possible that the analyte(s) in question are binding to suspended sediment in the samples, which would indicate matrix interference. This may account for the frequency of low matrix spike results in the January 2006 ELISA analyses. In the ELISAs conducted in 2007, matrix spike detection results were generally higher than expected, and exceeded the kits' upper detection limits. This indicates a limitation of the ELISA analysis that is not present with other methods of chemical analysis, such as GC-MS, which does not have an upper detection limit. With such a limitation, matrix interference appears to be a greater concern in that if a sample chosen as a matrix spike already has an analyte present, the results of that matrix spike may be further confounded. With the aforementioned limitations in mind, caution should be applied when interpreting ELISA data, especially when data are close to the detection limits.

Deviations: Eighteen deviations from the QA/QC plan occurred over the course of the project (see Table 11 below). These deviations included the following: exceeded holding time (68% or 13/19), exceeded sample temperatures (21% or 4/19), and other (11% or 2/19).

Exceeding sample holding time was the most frequent deviation. SWAMP requires that tests are to be initiated within 48 hours of sample collection. Instances in which holding time was missed generally consisted of samples being delayed or lost in the mail, unavailability of organisms for test initiation within the designated time frame, and technician oversight. Other reasons are as follows:

1. A *C. dubia* toxicity test did not meet test acceptability criteria and had to be re-set up. The retest was initiated past the 48-hour holding time.
2. The 48-hour holding time was exceeded in initiating a follow-up pathogen-related toxicity (PRT) test for *P. promelas*. Contractual requirements state that a follow-up PRT test must be initiated within 48-hours of observed toxicity. Statistical analyses had not been completed by that time, and the PRT test was initiated past the designated time frame.

3. Early in the project, samples were collected over several days and then shipped together, in some cases reaching UCD ATL after the last sample had been collected at the 48-hour mark. Holding time in those instances was thus already missed.

Corrective actions were initiated whenever possible. During periods of poor organism health, *C. dubia* samples were sent to AQUA-Science (Davis, CA) to complete testing in order to meet all test acceptability criteria. An improved time frame was devised in which statistics would be completed the day of test termination in order to meet any follow-up test holding time requirements. The DFG sampling crew devised a system in which samples collected prior to noon would be shipped the same day; samples collected after that time point would be shipped the next day, in order to meet test initiation holding time requirements. Technicians responsible for missing initial screening holding times were alerted to the problem, and meetings were held to promote contractual requirement awareness. During all instances stated above, holding time was exceeded by an average of 3.5 hours.

Warm sample receiving temperatures (> 6º C) was the second most frequent deviation. A corrective action was initiated with the first occurrence. The sampling agency was contacted and made aware of the problem. Additional ways to cool the samples were discussed, which included adding a plastic bag inside the coolers to hold more ice around the samples prior to shipping. These deviations occurred during the month of May for Region 7, and the warm sample temperatures were due to ice melting in the coolers during transit. As the sample receiving temperature was exceeded by an average of 1.4º C, loss of toxicity due to sample degradation is unlikely. Adding as much ice as possible that will fit inside the transport cooler prior to shipment may alleviate this deviation in the future.

Other deviations included a lack of biomass endpoint in a *P. promelas* test, and lack of final chemistry measurements in an *H. azteca* test. These deviations occurred due to technician oversight. Corrective actions were initiated with both deviations. The technicians in question were made aware of the problem and in the case of the missed biomass endpoint in the *P. promelas* test, a system was devised in order to keep better track of weighing schedules. However, the two samples (715CPVOD2 and 715CRIDG1) with the missed biomass endpoint in

the *P. promelas* test exhibited 100% survival and appeared healthy. The data is considered reliable.

Table 11. Deviations from the QA/QC plan

Region	Number of Deviations	Deviation Type	Frequency
3	4	Holding Time Exceeded	2
		Sample Temperature Exceeded	2
4	3	Holding Time Exceeded	3
7	7	Holding Time Exceeded	4
		Sample Temperature Exceeded	2
		No Biomass Endpoint in *P. promelas* test	1
9	5	Holding Time Exceeded	4
		No Final Chemistry Measurements in *H. azteca* test	1

Completeness: Completeness is a measure of the data obtained compared to the amount of data expected in a project. The toxicity data acquisition phase of a project is considered complete when all sites specified in a contract have been visited the number of times designated in a contract, the number of samples designated in a contract has been collected, and the number of toxicity tests and TIEs designated in the contract has been successfully completed. UCD ATL strives for a minimum of 90% completion of data. During the course of this project, 68 tests were conducted among four species, including any follow-up tests such as PRT toxicity testing or TIEs. Of those 68 tests, 65 met all test acceptability criteria. UCD ATL has achieved approximately 96% completion of data for this project.

SUMMARY AND DISCUSSION

Region 3

Among 59 water samples collected from 36 sites in RWQCB Region 3 and tested between January 1, 2006 and March 31, 2007, 28 samples (47.5%) collected from 25 sites (69.4% of sites) were toxic (Table 12).

Table 12. Toxic samples collected in Region 3 between January 1, 2006 and March 31, 2007.

Region 3 Site ID	*C. dubia* Survival	*C. dubia* Reproduction	*H. azteca* Survival	*P. promelas* Survival	*P. promelas* Biomass	*S. capricornutum* Cell Growth	TIE Result
304ARA001						1/3/06	
304BRA001						1/3/06	
304ZAY001		1/3/06					
304RIV001						1/3/06	
305SJN001		5/17/06				1/10/06[A]	
						5/17/06[A]	
305SAN001						1/10/06[A]	
						5/17/06[A]	
305CHI001						1/10/06	
305MUR001						1/10/06	
305COR001				1/10/06[B]	1/10/06[B]	1/10/06	
305CAR001		1/12/06					
305HAR001						1/12/06	
						5/30/06	
305WSA001						1/12/06	
305FDQ006		1/12/06				1/12/06	
305FUF001						5/17/06	
312CCC001						2/26/07[A]	
312NIT001						2/26/07	
312NIP001						2/26/07[A]	
312ALA001						2/26/07	
312BCD001	2/27/07	2/27/07				2/27/07	
312MSD001	2/27/07	2/27/07				2/27/07	
312MSS001	2/27/07	2/27/07		2/27/07	2/27/07	2/27/07	
312OFL001						2/28/07	
312ORC001	2/28/07	2/28/07				2/28/07	
312GVT001	2/28/07	2/28/07				2/28/07	
312ORB001	2/28/07	2/28/07				2/28/07	

A: Toxicity of this sample to *S. capricornutum* was likely to be caused by high conductivity
B: Toxicity seen after protocol was modified to control pathogens

The majority of toxic samples (44.1%) inhibited growth of green algae. Toxicity was likely caused by high conductivity in 6 of these samples (San Juan Creek at Anzar, San Benito at Y Road, Cuyama Creek d/s Cottonwood Canyon and Nipomo Creek). Of samples collected in February 2007, 84.6% were toxic to green algae. Two water samples collected on January 10, 2006 from Corralitos Creek (305COR001) and on February 27, 2007 from Main Street South (312MSS001) were acutely toxic to fathead minnow larvae. A total of 10 samples (17%) caused toxicity to *C. dubia.* Chronic toxicity (reproductive impairment) was seen in four samples collected in January and May 2006; two of them were not toxic to algae or fish, while two of them were also toxic to algae. Six of 13 samples (46.2%) collected in February 2007 were acutely toxic to *C. dubia.* All of these also inhibited algae growth.

In samples collected in January and May 2006, results of ELISA analysis (Appendix, Tables A32-35) of chlorpyrifos and diazinon showed concentrations at or close to detectable levels (<50 ng/L chlorpyrifos, <30 ng/L diazinon) of these OP pesticides in the samples. Because of technical limitations regarding the accuracy of the ELISA assays at low pesticide concentrations, we are unable to attribute the chronic *C. dubia* toxicity to these pesticides. It is, however, unlikely that OP pesticides were responsible for the widespread algae toxicity observed in these water samples. The cause(s) of the toxicity therefore remain unknown, but we recommend that the potential input of herbicides should receive some attention. In addition, green algae tend to be more sensitive to heavy metal toxicity than fathead minnow or *C.* dubia (Johnson et al., in review). Thus the potential influence of heavy metals should also be investigated.

Toxicity to *C. dubia* and possibly fathead minnow larvae observed in samples collected in February 2007 was mostly due to OP pesticides. Samples toxic to *C. dubia* contained approximately >3 to 8.5 TU chlorpyrifos and 0.7 to 2.4 TU diazinon. The toxicity of these two OP pesticides is additive (Bailey *et al*., 1997). It is highly likely that other toxicants were present, because of the high degree of algal toxicity in these samples.

Region 4

No toxicity was detected in the 12 water samples collected from Region 4.

Region 7

Of 9 samples collected from 9 sites in Region 7 in May 2006, two (22%) were toxic (Table 13). Site 723NRBDRY (New River at Boundary) exhibited acute toxicity to *H. azteca*. This site has a history of being toxic due to insecticides (see our 2006 report). The sample from site 719CVSC52 (Coachella Valley Storm Channel, Ave. 52) was acutely toxic to fish and chronically toxic to *C. dubia*. Further testing showed that toxicity was largely due to ammonia.

Table 13. Toxic samples collected in Region 7 between January 1, 2006 and March 31, 2007.

Region 7 Site ID	*C. dubia* Survival	*C. dubia* Reproduction	*H. azteca* Survival	*P. promelas* Survivial	*P. promelas* Biomass	TIE Result
723NRBDRY			5/1/06[A]			
719CVSC52		5/2/06		5/2/06	5/2/06	*P. promelas* toxicity was due to high ammonia.

A: 723NRBDRY 050106 Dilution series: **NOEC**: 6.25% **LOEC**: 12.5% **LC50**: 17.0%

Region 9

Table 14. Toxic samples collected in Region 9 between January 1, 2006 and March 31, 2007.

Region 9 Site ID	*C. dubia* Survival	*C. dubia* Reproduction	*H. azteca* Survival	*S. capricornutum* Cell Growth	TIE Result
908PPAR04				1/30/06	
909SSWR08				1/30/06	
911TTJR05			4/10/06	4/10/06	*H. azteca* toxicity was not due to ammonia
911TTET02	1/31/06	1/31/06		1/31/06	*C. dubia* toxicity was not due to ammonia
	4/11/06[A]	4/11/06		4/11/06	*C. dubia* toxicity was partially due to high ammonia and heavy metals.

A: 911TTET02 041106 Dilution series: **NOEC**: 50% **LOEC**: 100% **LC50**: 70.7%

Among the 9 sampling sites in Region 9, 4 sites (44.4%) exhibited algal toxicity, and one site (911TTET02, Tecate Creek 2) was toxic on two sampling dates in January and April 2006. Water from this site was also acutely toxic to *C. dubia*. Toxicity at sites 911TTJR05 (Tijuana

River 5) and 911TTET02 was not or only partially due to high ammonia levels. Heavy metals were contributing to the toxicity seen on 4/11/06 at site 911TTET02.

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