The University of California at Davis Aquatic Toxicology Laboratory (UCD ATL) stresses the importance of quality, in the work performed, as well as the data presented, and has adhered to stringent quality control procedures to ensure the accuracy of this report.

The data entry and statistical analyses are appropriate and compliant with UCD-ATL quality control guidelines.

Daniel Markiewicz, Statistician — Date: 4/11/06

The toxicity tests were performed according to the proper specifications. Deviations and/or corrective actions are documented in the data appendix.

Marie Vasi, Quality Assurance Manager — Date: 041106

The text of this report has been proofread and ensured to be correct.

Linda Deanovic, Laboratory Manager — Date: 041706

Inge Werner, PhD, Director — Date: 4-11-06

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**Surface Water Ambient Monitoring Program**

**State Water Resources Control Board**

**Contract #03-197-250-0**

# Toxicity Testing and Toxicity Identification Evaluation

## Final Report

**April 10, 2006**

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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 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. When samples caused 50% mortality within 96 h, toxicity identification evaluation tests (TIEs) were performed to identify the compound(s) causing toxicity.

Region 4: Among 65 water samples from sites in RWQCB Region 4 between April 25 and July 12, 2005, 21 samples (32%) collected from 18 sites (42% of sites) were toxic to *C. dubia*. Twelve of the sites in Region 4 where toxicity was detected were in the Los Angeles River watershed (LAR), while only six toxic sites were located in the San Gabriel River watershed (SGB) suggesting that toxicity may be more prevalent in the Los Angeles River watershed. Seven of 21 toxic samples were acutely toxic to *C. dubia*. Only one water sample (412 LARRHO; July 11, 2005) caused reduced survival and biomass in fathead minnow larvae in addition to *C. dubia* toxicity. Dilution series testing revealed that >16 toxic units (*C. dubia*) and 8 toxic units (fathead minnows) of contaminants were present in this sample, and TIE results showed that a mixture of chemicals was causing the observed toxicity. Cationic metals, in particular copper, aluminum, selenium, nickel and zinc, were identified as the dominant toxicants, in addition to nonpolar organic compound(s) and

volatile compounds (e.g. surfactants, ammonia, chlorine). A second water sample from Region 4 was (site 412LAR024, July 11, 2005) was subjected to TIE testing. Results indicated that non-polar organic compounds caused the toxicity, and that pyrethroid pesticides potentially contributed to the toxicity. Chemical analysis revealed the presence of two organophosphate insecticides, diazinon (0.05 ppb) and dioxathion (0.4 ppb).

Region 7: Of 15 sites sampled in Region 7 during 2004/2005, eight sites (53%) were toxic at least once during the sampling period. The Colorado River was toxic to *C. dubia* at the Nevada State Line and at the Imperial Dam Gates (sites 713CRNVBD, 715CRIDG1), and both sampling sites on the New River were acutely toxic to *H. azteca*. Test results on New River samples indicated that pyrethroid insecticides caused the toxicity. Chemical analysis did not detect pyrethroids in the May 2005 sample, but analysis of the October 2005 sample from this site revealed the presence of two pyrethroids at concentrations toxic to invertebrates: cyfluthrin (0.013 ppb) and permethrin (0.043 ppb). The detection of toxic concentrations of pyrethroid pesticides in the water column is of particular interest, because these compounds are believed to rapidly sequester to sediments and thus not cause toxicity in the water column. A sample taken from the Coachella Valley Stormchannel (719CVS52) was highly toxic to fathead minnow larvae, and caused reduced fecundity in *C. dubia*. The cause of toxicity was determined to be ammonia. Only one other site, Palo Verde Outfall Drain (715CPVOD2) showed acute fish toxicity as well as reduced fecundity in *C. dubia*. The cause of toxicity in this sample was not determined.

Region 9: Among the 22 sampling sites in Region 9, nineteen (86%) exhibited either invertebrate or algal toxicity, or both, in the period September 2004 - September 2005. At about half of them (nine sites) toxicity was measured more than once during the sampling period. Only three sites were exclusively toxic to algae: 907SDFRC2, 903SLMSA2 and 909SSWR08. The majority of water samples taken in April, May and June 2005 were solely toxic to *C. dubia*. Acute invertebrate toxicity was seen in two samples from site 911TTEC02, one of which was determined to be toxic due to ammonia and, in part, other unidentified toxicants.

# 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. Their Water Quality Control Plans are enforceable regulatory documents that outline toxicity standards and toxicity test compliance.

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 (Tasks 6 and 7) performed at the UCD ATL between June 1, 2004 and December 1, 2005 under agreement number 03-197-250 between UCD ATL and the California SWRCB.

# MATERIALS AND METHODS

## *Sampling Sites*

Sub-surface grab samples were collected by the State Department of Fish and Game, Moss Landing Marine Laboratories, and shipped to UCD ATL for toxicity tests. The sampling sites for each region are listed below (Tables 1-3).

Table 1: Sampling sites in Region 4 during 2004 and 2005.

| Station Name | Station ID | Latitude | Longitude |
|---|---|---|---|
| Los Angeles River Site 3 | 412LAR003 | 34.20105 | -118.30268 |
| Los Angeles River Site 4 | 412LAR004 | 34.26825 | -118.60806 |
| Los Angeles River Site 7 | 412LAR007 | 33.99431 | -118.18055 |
| Los Angeles River Site 8 | 412LAR008 | 34.26316 | -118.55704 |
| Los Angeles River Site 9 | 412LAR009 | 34.2273 | -118.31987 |
| Los Angeles River Site 13 | 412LAR013 | 34.29719 | -118.24705 |
| Los Angeles River Site 15 | 412LAR015 | 34.09369 | -118.02886 |
| Los Angeles River Site 16 | 412LAR016 | 34.15634 | -118.63878 |
| Los Angeles River Site 18 | 412LAR018 | 34.24375 | -118.22177 |
| Los Angeles River Site 19 | 412LAR019 | 34.15691 | -118.30350 |
| Los Angeles River Site 20 | 412LAR020 | 34.25773 | -118.59616 |
| Los Angeles River Site 23 | 412LAR023 | 33.86185 | -118.19653 |
| Los Angeles River Site 24 | 412LAR024 | 34.24862 | -118.54280 |
| Los Angeles River Site 25 | 412LAR025 | 34.25182 | -118.29597 |
| Los Angeles River Site 31 | 412LAR031 | 33.99525 | -118.10604 |
| San Gabriel River Site 3 | 405SGB003 | 34.281576 | -117.885085 |
| San Gabriel River Site 4 | 405SGB004 | 33.980641 | -117.929438 |
| San Gabriel River Site 6 | 405SGB006 | 34.255531 | -117.821396 |
| San Gabriel River Site 10 | 405SGB010 | 34.194981 | -117.86114 |
| San Gabriel River Site 11 | 405SGB011 | 33.821106 | -118.091579 |
| San Gabriel River Site 15 | 405SGB015 | 33.857834 | -118.111632 |
| San Gabriel River Site 18 | 405SGB018 | 34.140621 | -117.788428 |

| | | | |
|---|---|---|---|
| San Gabriel River Site 22 | 405SGB022 | 34.150193 | -117.837381 |
| San Gabriel River Site 25 | 405SGB025 | 34.127575 | -117.729001 |
| San Gabriel River Site 27 | 405SGB027 | 33.799587 | -118.090223 |
| San Gabriel River Site 34 | 405SGB034 | 34.224985 | -117.761116 |
| San Gabriel River Site 36 | 405SGB036 | 33.959357 | -117.9822 |
| San Gabriel River Site 41 | 405SGB041 | 34.12332 | -117.7725 |
| San Gabriel River Site 17 | 801SGB017 | 33.89618 | -117.79327 |
| San Gabriel River Site 7 | 845SGB007 | 33.89618 | -117.9054 |
| Morris Dam (downstream) | 405SGBMRS | 34.16933 | -117.88961 |
| San Gabriel Upper R2 | 405SGB065 | 34.27449 | -117.898 |
| San Gabriel Upper R1 | 405SGB042 | 34.1851 | -117.928 |
| Coldbrook | 405SGBVLD | 34.29216 | -117.839 |
| Verdugo Wash | 412LARVGO | 34.1546 | -118.2763 |
| Burbank Wash | 412LARBBK | 34.1599 | -118.3042 |
| Compton Creek | 412LARCMP | 33.8473 | -118.2096 |
| Rio Hondo | 412LARRHO | 33.9377 | -118.1707 |
| Arroyo Seco | 412LARSCO | 34.0827 | -118.2215 |
| Tujunga Wash | 412LARTJA | 34.1501 | -118.3907 |
| West Fork San Gabriel | 405SGBWFK | 34.2406 | -117.89 |
| North Fork San Gabriel | 405SGBNFK | 34.2482 | -117.8655 |
| East Fork San Gabriel | 405SGBEFK | 34.2379 | -117.8206 |
| Field Duplicate 17 – RWQCB4 | 400FDQ017 | 34.26316 | -118.55704 |

Table 2: Sampling sites in Region 7 during 2004 and 2005.

| Station Name | Station ID | Latitude | Longitude |
|---|---|---|---|
| Colorado River at Nevada State Line | 713CRNVBD | 34.4311 | -114.3095 |
| Palo Verde Lagoon (LG1) | 715CPVLG1 | 33.43627 | -114.716 |
| Palo Verde Outfall Drain (PVOD2) | 715CPVOD2 | 33.4226 | -114.7262 |
| Palo Verde Drain | 715 CPVDRN | 33.43695 | -114.7303 |
| Colorado River at Imperial Dam Grates | 715CRIDG1 | 32.88482 | -114.4677 |
| Alamo River Outlet | 723ARGRB1 | 33.1992 | -115.5971 |
| Alamo River at International Boundary | 723ARINTL | 32.67506 | -115.3701 |
| New River at Boundary | 723NRBDRY | 32.66583 | -115.5022 |
| New River Outlet | 723NROTWM | 33.10472 | -115.6636 |
| Salton Sea Drain NW2 (Torrez Martinez 2) | 728SSDNW2 | 33.45067 | -115.9947 |
| Salton Sea USGS2 | 728SSGS02 | 33.23333 | -115.75 |
| Salton Sea USGS7 | 728SSGS07 | 33.325 | -115.8111 |
| Salton Sea USGS9 | 728SSGS09 | 33.4 | -115.925 |
| Coachella Valley Stormchannel (Ave 52) | 719CVSC52 | 33.67242 | -116.1492 |
| Coachella Valley Stormwater Channel Outlet | 719CVSCOT | 33.52444 | -116.0778 |
| Field Duplicate 10 – RWQCB7 | 700FDQ010 | 33.43627 | -114.716 |
| Field Duplicate 11 – RWQCB7 | 700FDQ011 | 33.1992 | -115.5971 |
| Field Duplicate 12 – RWQCB7 | 700FDQ012 | 33.10472 | -115.6636 |

Table 3: Sampling sites in Region 9 during 2004 and 2005.

| Station Name | Station ID | Latitude | Longitude |
|---|---|---|---|
| Gird Creek 2 | 903SLGRD2 | 33.33549 | -117.18845 |
| Iron Springs Creek 2 | 903SLIRS2 | 33.33326 | -116.87164 |
| Keys Creek 3 | 903SLKYS3 | 33.28908 | -117.07136 |
| Moosa Creek 2 | 903SLMSA2 | 33.28559 | -117.20873 |
| San Luis Rey River 2 | 903SLSLR2 | 33.26329 | -116.8089 |
| San Luis Rey River 8 | 903SLSLR8 | 33.21495 | -117.36838 |
| Alvarado Creek 3 | 907SDALV3 | 32.78309 | -117.07478 |
| Boulder Creek 2 | 907SDBOC2 | 32.96353 | -116.66406 |
| Forrester Creek 2 | 907SDFRC2 | 32.83945 | -117.00107 |
| Los Coches Creek 2 | 907SDLCO2 | 32.84914 | -116.85907 |
| San Vicente Creek 4 | 907SDSVC4 | 32.99342 | -116.84979 |
| San Diego River 15 | 907SSDR15 | 32.76194 | -117.1927 |
| Tijuana River 5 | 911TTJR05 | 32.54919 | -117.06514 |
| Sweetwater River 3 | 909SSWR03 | 32.83521 | -116.62203 |
| Sweetwater River 8 | 909SSWR08 | 32.65897 | -117.04181 |
| La Posta Creek 4 | 911TLAP04 | 32.69997 | -116.47959 |
| Creek 2 | 908SLAW02 | 32.75409 | -116.77885 |
| La Posta Creek 4 | 900FDQ014 | 32.69997 | -116.47959 |
| 10 | 911TCWD10 | 32.573 | -116.75753 |
| Tecate Creek 2 | 911TTEC02 | 32.56539 | -116.7585 |
| Creek 2 | 908PTEL02 | 32.62853 | -117.05751 |
| Paradise Creek 4 | 908PPAR04 | 32.66943 | -117.10279 |
| Field Duplicate 10 – RWQCB9 | 900FDQ010 | 33.33549 | -117.18845 |
| Field Duplicate 12 - RWQCB9 | 900FDQ012 | 33.28908 | -117.07136 |
| Field Duplicate 14 – RWQCB9 | 900FDQ014 | 32.69997 | -116.47959 |
| Field Duplicate 15 – RWQCB9 | 900FDQ015 | 32.69997 | -116.47959 |

## *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. The toxicity tests during the September-December 2004 period were performed at AQUA-Science, Davis, CA. Summaries of the testing procedures are provided below.

Table 4. Toxicity tests performed on samples from Region 4 during 2004/05.

| Station Name | *Ceriodaphnia dubia* | *Selenastrum capricornutum* | *Hyalella azteca* | *Pimephales promelas* |
|---|---|---|---|---|
| Los Angeles Random Site 3 | x | | | x |
| Los Angeles Random Site 4 | x | | | x |
| Los Angeles Random Site 7 | x | | | x |
| Los Angeles Random Site 8 | x | | | x |
| Los Angeles Random Site 9 | x | | | x |
| Los Angeles Random Site 13 | x | | | x |
| Los Angeles Random Site 15 | x | | | x |
| Los Angeles Random Site 16 | x | | x* | x |
| Los Angeles Random Site 18 | x | | | x |
| Los Angeles Random Site 19 | x | | | x |
| Los Angeles Random Site 20 | x | | | x |
| Los Angeles Random Site 23 | x | | | x |
| Los Angeles Random Site 24 | x | | | x |
| Los Angeles Random Site 25 | x | | | x |
| Los Angeles Random Site 31 | x | | | x |
| San Gabriel Random Site 3 | x | | | x |
| San Gabriel Random Site 4 | x | | | x |
| San Gabriel Random Site 6 | x | | | x |
| San Gabriel Random Site 10 | x | | | x |
| San Gabriel Random Site 11 | x | | | x |
| San Gabriel Random Site 15 | x | | | x |
| San Gabriel Random Site 18 | x | | | x |
| San Gabriel Random Site 22 | x | | | x |
| San Gabriel Random Site 25 | x | | | x |
| San Gabriel Random Site 27 | x | | | x |
| San Gabriel Random Site 34 | x | | | x |
| San Gabriel Random Site 36 | x | | x** | x |
| San Gabriel Random Site 41 | x | | | x |
| San Gabriel Random Site 17 | x | | | x |

| | | | | |
|---|---|---|---|---|
| San Gabriel Random Site 7 | x | | | x |
| Morris Dam (downstream) | x | | | x |
| San Gabriel Upper R2 | x | | | x |
| San Gabriel Upper R1 | x | | | x |
| Coldbrook | x | | | x |
| Verdugo Wash | x | | | x |
| Burbank Wash | x | | | x |
| Compton Creek | x | | | x |
| Rio Hondo | x | | | x |
| Arroyo Seco | x | | | x |
| Tujunga Wash | x | | | x |
| West Fork San Gabriel | x | | | x |
| North Fork San Gabriel | x | | | x |
| East Fork San Gabriel | x | | | x |
| Field Duplicate 17 – RWQCB4 | x | | | x |

* during June 2005 this site was tested with *H. azteca*
** during May 2005 this site was tested with *H. azteca*

Table 5. Toxicity tests performed on samples from Region 7 during 2004/05.

| Station Name | *Ceriodaphnia dubia* | *Atherinops affinis* | *Hyalella azteca* | *Pimephales promelas* |
|---|---|---|---|---|
| Colorado River at Nevada State Line | x | | | x |
| Palo Verde Lagoon (LG1) | x | | | x |
| Palo Verde Outfall Drain (PVOD2) | x | | | x |
| Palo Verde Drain | x | | | |
| Colorado River at Imperial Dam Grates | x | | | x |
| Alamo River Outlet | | | x | x |
| Alamo River at International Boundary | x* | | x | x |
| New River at Boundary | | | x | |
| New River Outlet | | | x | |
| Salton Sea Drain NW2 (Torrez Martinez 2) | | x | | |
| Salton Sea USGS2 | | x | | |
| Salton Sea USGS7 | | x | | |
| Salton Sea USGS9 | | x | | |
| Coachella Valley Stormchannel (Ave 52) | x | | | x |
| Coachella Valley Stormwater Channel Outlet | x | | | x |
| Field Duplicate 10 – RWQCB7 | x | | | x |
| Field Duplicate 11 – RWQCB7 | | | x | x |
| Field Duplicate 12 – RWQCB7 | | | x | |

* during May 2005 this site was tested with *H. azteca*

Table 6. Toxicity tests performed on samples from Region 9 during 2004/05.

| Station Name | *Ceriodaphnia dubia* | *Selenastrum capricornutum* | *Hyalella azteca* | *Pimephales promelas* |
|---|---|---|---|---|
| Gird Creek 2 | x | x | | |
| Iron Springs Creek 2 | x | x | | |
| Keys Creek 3 | x | x | | |
| Moosa Creek 2 | x | x | | |
| San Luis Rey River 2 | x | x | | |
| San Luis Rey River 8 | x | x | | |
| Alvarado Creek 3 | x | x | x | |
| Boulder Creek 2 | x | x | | |
| Forrester Creek 2 | x | x | x | |
| Los Coches Creek 2 | x | x | | |
| San Vicente Creek 4 | x | x | | |
| San Diego River 15 | x | x | | |
| Tijuana River 5 | x | x | | |
| Sweetwater River 3 | x | x | | |
| Sweetwater River 8 | x | x | x | |
| La Posta Creek 4 | | x | x | |
| Creek 2 | x | x | | |
| La Posta Creek 4 | x | x | | |
| 10 | x | x | | |
| Tecate Creek 2 | | x | x | |
| Creek 2 | x | x | | |
| Paradise Creek 4 | x | x | x | |
| Field Duplicate 10 – RWQCB9 | x | x | | |
| Field Duplicate 12 - RWQCB9 | x | x | | |
| Field Duplicate 14 - RWQCB9 | x | x | | |
| Field Duplicate 15 – RWQCB9 | | x | x | |

## *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$^{TM}$ 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. Each replicate is fed 100 µl of a 2:1 *Selenastrum*:YCT mixture two hours before renewal on day 5. 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 x $10^6$ 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 cool white fluorescent light with a 16-hour light: 8-hour dark photoperiod. 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).

***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. TUs are estimated by dividing the undiluted sample by the lowest sample dilution causing toxicity. For example, if the sample diluted to 25% causes toxicity, the sample contains at least 4 TUs of toxic substances. TUs contributed by individual toxic chemicals can also be estimated. In this context, 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. 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 include manipulations including,

but not limited to, air-stripping, Disodium Ethylenediamine Tetraacetate (EDTA) addition, Sodium Thiosulfate (STS) addition, Piperonyl Butoxide (PBO) addition, and solid phase extraction (C8-SPE).

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 are added to the toxic samples for a final concentration of 0.1 ppm (*C. dubia, H. azteca*). 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 and the through-column "rinsate" is tested along with the unmanipulated sample. Control water also is passed through an SPE column and serves as one of the method controls (blank). The adsorbate is then eluted with methanol and the eluate added to control water and tested along with the appropriate method control. If the toxicant is a non-polar organic chemical, the ambient sample and control water amended with methanol eluate will exhibit mortality while the sample passed through the SPE column results in 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, 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.

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 high ammonia measurements, the pH of the water sample was adjusted to 7.3 and 6.3. At lower pH levels ammonia ($NH_3$) is converted to ionic ammonium ($NH_4^+$), which is less toxic to aquatic organisms, therefore a reduction in toxicity due to lowering of the pH confirmed that ammonia was responsible for the observed toxicity.

Phase II TIEs are conducted on toxic samples at the Contract Manager's request to tentatively identify the suspected toxicant(s) in the sample. Results furnished by acute or chronic Phase I tests usually give guidance in achieving this goal. When Phase I TIEs implicated non-polar organic compounds in all samples tested, Phase II TIEs focused on this class of chemicals. Varian or Baker C8-SPE columns (6 ml) were used to extract non-polar organic chemicals from the water sample. Both control and sample waters (1000-1800 ml) were pumped through C8-SPE columns (5-10 ml/min). The initial 200 ml of filtered water were discarded to minimize potential artifactual column toxicity; the remaining filtrate was subject to toxicity testing (*C. dubia* or *H. azteca*). Columns were eluted with 9 increasing concentrations of HPLC grade methanol (3 ml at 1 ml/min) to sequentially remove non-polar organic compounds of decreasing polarities. Each resultant fraction was diluted in control water to 0.5% extract and tested. Solvent controls contained 0.5% methanol.

## *Chemical Analyses*

Analyses were performed by the State Department of Fish and Game: Nimbus Laboratory, Rancho Cordova, CA. Whole water sub-samples of a toxic sample to be analyzed for organics were homogenized and aliquoted. Whole water sub-samples of a toxic sample to be analyzed for total metals were homogenized, aliquoted and preserved in HNO3. Sub-samples of a toxic sample to be analyzed for dissolved metals were homogenized, aliquoted, filtered through a 0.20µm nylon filter and preserved in HNO3. Phase I TIE results determined which type of chemical analysis was performed.

The following samples were submitted for chemical analyses:

Region 4: 412LARRHO, sample date 071105; requested dissolved and total metals analysis on a 4-liter sample.

Region 4: 412LAR024, sample date 071105; requested pesticide scan on a 4-liter sample.

Region 7: 723NRBDRY, sample date 050905; requested pyrethroid scan on a 4-liter sample and column eluate.

Region 7: 723NRBDRY, sample date 102505; requested pesticide scan - SPE column.

## *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 15$^{th}$ 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 is presented in Appendix A.

## *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.

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

## September 30 – December 31, 2004

### Region 7

The results of the *C. dubia* chronic toxicity tests conducted on samples collected on October 4-5, 2004 are summarized in Table 7. The conductivity of 7 of the 9 samples exceeded 2000µS and was adjusted to approximately 2000µS prior to test initiation. The samples were tested along with a 2000µS conductivity control and two laboratory water controls. Sample ARGRB1 exhibited 100% mortality within 24 hours. Sample 713CRNVBD exhibited statistically significant ($p<0.05$) mortality (30% mortality by day 5) and statistically significant ($p<0.05$) reduction in reproduction. Follow-up testing was requested for ARGRB1.

A dilution series was conducted and it was determined that the sample had a 48-hour EC50 of 9.2%, and that approximately 10.9 toxic units (TU) were present in the sample. ELISA analysis of the sample detected 157 ng/L chlorpyrifos (2.0 TU) and 1655 µg/L of diazinon (4.4 TU). Chlorpyrifos and diazinon, both organophosphorous (OP) insecticides, accounted for approximately 60% (6.4 TU) of the toxicity in the sample. A Phase I TIE was conducted and piperonyl butoxide (PBO) reduced toxicity in the sample, confirming that toxicity was due to metabolically activated OPs.

Table 7: Summary of *C. dubia* Chronic Toxicity Tests for Region 7
These tests were performed at AQUA-Science, Davis, CA.

| Sample ID | Sampling Date | Mortality (%) | Reproduction (Neonates/Female) |
|---|---|---|---|
| EC Control[a] | -- | 0 | 29.5 |
| ARGRB1 | 10/5/04 | 100* | 0* |
| CVSCOT | 10/5/04 | 10 | 27.7 |
| FDQ010 | 10/5/04 | 20 | 23.4 |
| ARINTL | 10/4/04 | 0 | 34.1 |
| 715CPVDRN | 10/5/04 | 0 | 35.8 |
| 715CPVOD2 | 10/5/04 | 0 | 33.0 |
| 715CPVLG1 | 10/5/04 | 0 | 33.3 |
| Control 1[b] | -- | 0 | 31.8 |
| 713CRNVBD | 10/4/04 | 30* | 17.2* |
| Control 2[c] | -- | 0 | 30.7 |
| 715CRIDG1 | 10/5/04 | 10 | 32.1 |

*Significantly different than control ($p<0.05$)
a EC control was EPA moderately hard water adjusted to ~2000µS using seawater
b Control 1 was compared with 713CRNVBD
c Control 2 was compared with 715CRIDG1

***Water Chemistry***

Water quality data is presented in detail in Appendix A. Prior to *C. dubia* and *S. capricornutum* test initiation, temperature, dissolved oxygen (DO), pH, alkalinity, hardness, electrical conductivity (EC), and ammonia were measured, and all were within the physiological limits of the test species. For *S. capricornutum* test temperature, DO, and pH were measured daily, and were also measured at test termination, in addition to EC, ensuring that water chemistry remained within physiological limits. In the *C. dubia* tests, temperature, DO, pH, and EC were measured in the daily renewal water and the 24-hour old water ensuring that water chemistry remained within physiological limits.

## Region 9

Samples from Region 9 were collected on September 13-14, 2004. The toxicity tests were performed at AQUA-Science, Davis, CA.

### *Ceriodaphnia dubia*

The results of the *C. dubia* chronic toxicity tests conducted on samples collected on September 13-14, 2004 are summarized in Table 8. The conductivity of 10 of the 12 samples exceeded 2000µS and was adjusted to approximately 2000µS prior to test initiation. The samples were tested along with a 2000µS conductivity control and two laboratory water controls.

Table 8: Summary of *C. dubia* Toxicity Tests for Region 9.
These tests were performed at AQUA-Science, Davis, CA.

| Sample ID | Sampling Date | Mortality (%) | Reproduction (Neonates/female) |
|---|---|---|---|
| EC Control[a] | -- | 0 | 32.6 |
| 907SSDR15 | 9/13/04 | 0 | 33.6 |
| 907SDFRC2 | 9/13/04 | 0 | 33.8 |
| 907SDALV3 | 9/13/04 | 10 | 32.7 |
| 907SDLC02 | 9/13/04 | 0 | 23.3* |
| 907SDSVC4 | 9/13/04 | 0 | 28.5 |
| 903SLGRD2 | 9/14/04 | 0 | 12.1* |
| 903SLSLR8 | 9/14/04 | 0 | 28.3* |
| 903SLMSA2 | 9/14/04 | 10 | 28.4 |
| 903SLKYS3 | 9/14/04 | 10 | 20.6* |
| 900FDQ010 | 9/14/04 | 20 | 7.9* |
| Control 1[b] | -- | 10 | 36.1 |
| 903SLSLR2 | 9/13/04 | 0 | 28.8* |
| Control 2[c] | -- | 0 | 38.3 |
| 903SLIRS2 | 9/13/04 | 0 | 35.0* |

*Significantly different than control ($p<0.05$)
a EC control was EPA moderately hard water adjusted to ~2000µS using seawater
b Control 1 was compared with 903SLSLR2
c Control 2 was compared with 903SLIRS2

There were no statistically significant effects on survival. However, several samples exhibited a statistically significant reduction ($p<0.05$) in reproduction compared to the control. These were samples from sites: 907SDLC02, 903SLSLR2, 903SLIRS2, 903SLGRD2, 903SLSLR8, 903SLKYS3, and 900FDQ010.

***Selenastrum capricornutum***

The results of the *S. capricornutum* chronic toxicity tests conducted on samples collected on September 13-14, 2004 are summarized in Table 9. The samples were not conductivity-adjusted prior to algae test initiation. All of the samples, except for 903SLIRS2, exhibited a statistically significant reduction ($p<0.05$) in cell growth from the control.

Table 9: Summary of *S. capricornutum* chronic toxicity tests for Region 9. The tests were performed at AQUA-Science, Davis, CA.

| Sample ID | Sampling Date | Algae Cell No. x $10^6$ |
|---|---|---|
| Control 1 | -- | 3.96 |
| 907SSDR15 | 9/13/04 | 0.61* |
| 907SDFRC2 | 9/13/04 | 2.94* |
| 907SDALV3 | 9/13/04 | 2.20* |
| 907SDLC02 | 9/13/04 | 1.93* |
| Control 2 | -- | 3.83 |
| 907SDSVC4 | 9/13/04 | 2.33* |
| 903SLSLR2 | 9/13/04 | 3.34* |
| 903SLIRS2 | 9/13/04 | 4.20 |
| 903SLGRD2 | 9/14/04 | 1.88* |
| Control 3 | -- | 3.50 |
| 903SLSLR8 | 9/14/04 | 0.40* |
| 903SLMSA2 | 9/14/04 | 1.63* |
| 903SLKYS3 | 9/14/04 | 1.02* |
| 900FDQ010 | 9/14/04 | 1.85* |

* Significantly different than control ($p<0.05$)

## January 1 - March 31, 2005

UCD ATL performed all but one of the toxicity tests on samples collected on February 28, 2005 and March 1, 2005 for Region 9. The sample collected February 28, 2005 at station 907SDFRC2 had an electrical conductivity that exceeded 2500 µmhos (exceeding the tolerance of *Ceriodaphnia dubia*). The State Water Resources Control Board (SWRCB) requested that samples not be diluted, so the sample was tested with *Hyalella azteca (H. azteca)* at UCD Granite Canyon Laboratory.

**Region 9**

***Ceriodaphnia dubia***

The results of the *C. dubia* chronic toxicity test conducted on samples collected on February 28, 2005 and March 1, 2005 are summarized in Table 10.

The conductivity of the sample collected on February 28, 2005 at station 907SDFRC2 exceeded 2500 µS and was sent to Granite Canyon Laboratory for testing with *Hyalella azteca.* There were no statistically significant effects on survival. However, several samples exhibited a statistically significant reduction ($p<0.05$) in reproduction from the control. These were from sites: 907SDALV3, 907SDSVC4, and 903SLGRD2.

The results of the *C. dubia* chronic toxicity test conducted on samples collected on March 1-2, 2005 are summarized in Table 11. There were no statistically significant effects on survival or reproduction.

Table 10: Summary of 7-day *C. dubia* toxicity test conducted on samples collected for Region 9 on 2/28/05 – 3/01/05.[1]

| Treatment | Reproduction[2] | | Mortality[2] (%) |
|---|---|---|---|
| | x | se | |
| Laboratory Control (SSEPAMH) | 26.1[P] | 0.95 | 0.0[P] |
| 907SDR15 | 24.0 | 1.91 | 0.0 |
| 907SDALV3 | 17.8* | 1.00* | 0.0 |
| 907SDSVC4 | 22.0* | 1.39* | 0.0 |
| 907SDLCO2 | 25.1 | 1.85 | 0.0 |
| 903SLMSA2 | 22.8 | 1.50 | 0.0 |
| 907SDCHC3 | 25.0 | 1.59 | 0.0 |
| 903SLSLR2 | 30.3 | 0.47 | 0.0 |
| 903SLIRS2 | 20.2 | 3.44 | 10.0 |
| 903SLGRD2 | 8.3* | 1.13* | 0.0 |
| 907SDBOC2 | 24.3 | 1.83 | 0.0 |
| 907HDLIC4-C | 25.6 | 1.32 | 0.0 |

P. The laboratory control met all EPA criteria for test acceptability. 100% of the daphnids had a third brood.
1. This test was set up on 3/02/05.
2. * indicates a significant reduction in reproduction or increase in mortality relative to the laboratory control water. The mortality endpoint was analyzed using Fisher's Exact Test. The reproductive endpoint was analyzed using Dunnett's Test ($p<0.05$) or Bonferroni corrected Wilcoxan tests.

Table 11: Summary of 7-day *C. dubia* toxicity test conducted on samples collected for Region 9 on 3/01/05 – 3/02/05.[1]

| Treatment | Reproduction[2] | | Mortality[2] (%) |
|---|---|---|---|
| | x | se | |
| Laboratory Control (SSEPAMH) | 21.1[P] | 0.58 | 0.0[P] |
| 903SLSLR8 | 24.8 | 0.90 | 0.0 |
| 903SLKYS3 | 20.6 | 0.76 | 0.0 |
| 900FDQ012 | 21.0 | 0.84 | 0.0 |

P. The laboratory control met all EPA criteria for test acceptability. 100% of the daphnids had a third brood.
1. This test was set up on 3/03/05.
2. Highlighted cells indicate a significant reduction in reproduction or increase in mortality relative to the laboratory control water. The mortality endpoint was analyzed using Fisher's Exact Test. The reproductive endpoint was analyzed using Dunnett's Test ($p<0.05$) or Bonferroni corrected Wilcoxan tests.

***Selenastrum capricornutum***

The results of the *S. capricornutum* chronic toxicity test conducted on samples collected on February 28, 2005 and March 1, 2005 are summarized in Table 12. High EC controls (glass distilled water adjusted to 2000 or 3000 µmhos with seawater) were included in the test for comparison with high EC samples. Four samples had an EC between 1500 and 2500 µmhos and were compared to the 2000 µmhos high EC control. The rest of the samples were compared to the glass distilled control. The sample collected at 903SLGRD2 caused a statistically significant reduction ($p<0.05$) in cell growth.

The results of the *S. capricornutum* chronic toxicity test conducted on samples collected on March 1-2, 2005 are summarized in Table 13. High EC controls were included for comparison with high EC samples. Two samples had an EC between 1500 and 2500 µmhos and were compared to the 2000 µmhos high EC control. The third sample was compared to the glass distilled control. None of the samples exhibited a statistically significant reduction ($p<0.05$) in cell growth from the control.

***Hyalella azteca***

The sample collected on February 28, 2005 at station 907SDFRC2 was tested at Granite Canyon Laboratory with *Hyalella azteca.* There were no statistically significant effects on survival.

***Water Chemistry***

Detailed water quality data is presented in Appendix A. Prior to *C. dubia* and *S. capricornutum* test initiation and at test termination, temperature, dissolved oxygen (DO), pH, electrical conductivity (EC), alkalinity, hardness, and ammonia were measured. During the *S. capricornutum* test pH was measured daily. In the *C. dubia* tests, DO was measured in the daily renewal water, and DO and pH were measured in the 24-hour old water. Temperature was monitored continuously throughout all toxicity tests. Water chemistry was within physiological limits throughout all of the tests.

Table 12. Summary of 96-hr *S. capricornutum* toxicity test conducted on samples collected for Region 9 on 2/28/05 and 3/01/05.

| Treatment | Cell Count ($6^2$) | | % CV | Final pH at 96 hrs |
|---|---|---|---|---|
| | x | se | | |
| Laboratory Control (Glass Distilled) | 1.815[P] | 0.1 | 17.8 | 9.08 |
| Laboratory Control (Glass Distilled + Seawater @ 2000 | 2.133 | 0.1 | 14.4 | 8.86 |
| Laboratory Control (Glass Distilled + Seawater @ 3000 | 1.954 | 0.2 | 18.8 | 8.81 |
| 907SDR15 | 2.080 | 0.1 | 9.2 | 9.08 |
| 907SDALV3 [3] | 1.804 | 0.3 | 37.8 | 8.73 |
| 907SDSVC4 | 2.018 | 0.4 | 36.6 | 9.14 |
| 907SDFRC2 [3] | 1.967 | 0.3 | 27.2 | 8.62 |
| 907SDLCO2 | 2.057 | 0.3 | 26.7 | 9.18 |
| 903SLMSA2 | 2.158 | 0.3 | 24.7 | 9.09 |
| 907SDCHC3 | 2.093 | 0.2 | 19.8 | 8.93 |
| 903SLSLR2 | 2.172 | 0.2 | 16.2 | 9.32 |
| 903SLIRS2 [3] | 1.906 | 0.2 | 23.6 | 9.21 |
| 903SLGRD2[3] | 1.366 | 0.1 | 17.6 | 8.42 |
| 907SDBOC2 | 2.094 | 0.3 | 26.1 | 9.34 |
| 907HDLIC4-A | 2.148 | 0.2 | 15.0 | 9.31 |

1. This test was set up on 3/02/05.
2. Highlighted areas indicate a significant reduction in cell count compared to the laboratory control. The cell count endpoint was analyzed using Dunnett's Test ($p<0.05$) or Bonferroni corrected Wilcoxan tests.
3. Samples with EC between 1500 - 2500 (before adding nutrients) were compared to the 2000 µmhos EC control water.

Table 13. Summary of 96-hr *S. capricornutum* toxicity test conducted on samples collected for Region 9 on 3/01/05-3/02/05.

| Treatment | Cell Count ($6^2$) | | % CV | Final pH at 96 hrs |
|---|---|---|---|---|
| | x | se | | |
| Laboratory Control (Glass Distilled) | 1.771[P] | 0.1 | 18.6 | 9.18 |
| Laboratory Control (Glass Distilled + Seawater) @ 2000 [1] | 1.432 | 0.1 | 13.5 | 9.09 |
| 903SLSLR8 | 1.473 | 0.2 | 24.2 | 8.99 |
| 903SLKYS3 [3] | 1.214 | 0.2 | 32.0 | 8.68 |
| 900FDQ012 [3] | 1.315 | 0.1 | 21.5 | 8.70 |

1. This test was set up on 3/03/05.
2. Highlighted areas indicate a significant reduction in cell count compared to the laboratory control. The cell count endpoint was analyzed using Dunnett's Test ($p<0.05$) or Bonferroni corrected Wilcoxan tests.
3. Samples with EC between 1500 - 2500 (before adding nutrients) were compared to the high EC control water.

## April 1- June 30, 2005

**Region 4**

***Ceriodaphnia dubia***

The results of the *C. dubia* chronic toxicity tests conducted on samples collected between April 25, 2005 and June 16, 2005 are summarized in Table 14. Eleven samples exhibited a statistically significant reduction ($p<0.05$) in reproduction as compared to the control. These were: 412LAR008, 412LAR024, 412LAR025 (collected April 27, 2005), 412LAR009, 412LAR020, 412LAR025 (collected May 16, 2005), 405SGB022, 405SGB015 pH shifted, 412LAR023 pH shifted, 412LARVGO, and 405SGBNFK. Two samples caused a significant increase in mortality as compared to the control: 412LAR025 (collected April 27, 2005) and 412LARVGO.

***Pimephales promelas***

The results of the *P. promelas* chronic toxicity tests conducted on samples collected between April 25, 2005 and June 16, 2005 are summarized in Table 15. There were no statistically significant reductions in biomass or survival in any of the tests.

***Hyalella azteca***

The results of the *H. azteca* chronic toxicity test conducted on a Region 4 sample collected on May 25, 2005 are shown in Tables 16. There was no statistically significant reduction in survival.

Table 14. Summary of 7-day *C. dubia* toxicity test conducted on samples collected for Region 4 between April 25, 2005 and June 16, 2005.

| Sample ID | Sampling Date | Survival (%) | Reproduction (neonates/female) |
|---|---|---|---|
| SSEPAMH | Laboratory Control | 100 | 22.4 |
| 412LAR004 | 4/26/2005 | 100 | 19.1 |
| 412LAR020 | 4/26/2005 | 100 | 21.0 |
| 412LAR008 | 4/26/2005 | 100 | 15.7* |
| 412LAR024 | 4/26/2005 | 70 | 0* |
| 400FDQ017 | 4/26/2005 | 90 | 17.0 |
| SSEPAMH | Laboratory Control | 100 | 19.1 |
| 412LAR03 | 4/27/2005 | 100 | 17.1 |

| | | | |
|---|---|---|---|
| 412LAR019 | 4/27/2005 | 100 | 17.9 |
| 412LAR025 | 4/27/2005 | 10* | 0* |
| SSEPAMH | Laboratory Control | 90 | 24.1 |
| 412LAR009 | 5/16/2005 | 90 | 15.8* |
| 412LAR020 | 5/16/2005 | 100 | 17.4* |
| 412LAR025 | 5/16/2005 | 50 | 0.0* |
| 405SGB022 | 5/17/2005 | 100 | 14.4* |
| SSEPAMH | Laboratory Control | 90 | 17.5 |
| 405SGB025 | 5/17/2005 | 100 | 20.5 |
| 405SGB025 pH shift | 5/17/2005 | 80 | 21.2 |
| 405SGB004 | 5/18/2005 | 100 | 18.0 |
| 405SGB018 | 5/18/2005 | 100 | 17.3 |
| SSEPAMH | Laboratory Control | 100 | 24.9 |
| 412LAR015 | 5/23/2005 | 90 | 20.2 |
| 412LAR018 | 5/23/2005 | 90 | 27.2 |
| SSEPAMH | Laboratory Control | 95 | 22.8 |
| 405SGB027 | 5/24/2005 | 90 | 18.9 |
| 801SGB017 | 5/24/2005 | 100 | 20.1 |
| 405SGB015 | 5/24/2005 | 100 | 21.0 |
| 412LAR023 | 5/24/2005 | 90 | 28.0 |
| 405SGB015 pH shift | 5/24/2005 | 90 | 4.8* |
| 412LAR023 pH shift | 5/24/2005 | 90 | 12.7 |
| SSEPAMH | Laboratory Control | 100 | 23.5 |
| 845SGB007 | 5/25/2005 | 90 | 20.4 |
| 405SGB011 | 5/25/2005 | 100 | 19.7 |
| SSEPAMH | Laboratory Control | 100 | 26.5 |
| 405SGB034 | 6/7/2005 | 100 | 20.2 |
| 405SGB041 | 6/7/2005 | 90 | 26.7 |
| 405SGB041 pH shift | 6/7/2005 | 100 | 27.7 |
| SSEPAMH | Laboratory Control | 88.9 | 22.4 |
| 405SGB010 | 6/8/2005 | 100 | 22.4 |
| 405SGBMRS | 6/9/2005 | 100 | 21.5 |
| SSEPAMH | Laboratory Control | 100 | 27.0 |
| 412LARVGO | 6/13/2005 | 0* | 0.0* |
| 412LARBBK | 6/13/2005 | 100 | 29.9 |
| SSEPAMH | Laboratory Control | 100 | 26.6 |
| 405SGB003 | 6/14/2005 | 100 | 23.9 |
| SSEPAMH | Laboratory Control | 95 | 21.1 |
| 405SGBWFK | 6/15/2005 | 100 | 20.4 |
| 405SGBNFK | 6/15/2005 | 100 | 4.1* |
| 405SGBEFK | 6/15/2005 | 100 | 17.4 |
| 400FDQ016 | 6/15/2005 | 100 | 18.5 |
| SSEPAMH | Laboratory Control | 100 | 24.0 |
| 405SGB006 | 6/16/2005 | 60 | 2.5* |

*Significantly different from control ($p<0.05$).

Table 15. Summary of chronic *P. promelas* toxicity tests conducted on samples collected for Region 4 between April 25, 2005 and June 16, 2005. * significantly different from control ($p<0.05$)

| Sample ID | Sampling Date | Biomass (mg/individual) | Survival (%) |
|---|---|---|---|
| DIEPAMH | Laboratory Control | 0.252 | 93.8 |
| 412LAR004 | 4/26/2005 | 0.260 | 92.5 |
| 412LAR020 | 4/26/2005 | 0.215 | 75.0 |
| 412LAR008 | 4/26/2005 | 0.277 | 90.0 |
| 412LAR024 | 4/26/2005 | 0.280 | 100.0 |
| 400FDQ017 | 4/26/2005 | 0.295 | 95.0 |
| DIEPAMH | Laboratory Control | 0.307 | 92.5 |
| 412LAR03 | 4/27/2005 | 0.357 | 92.5 |
| 412LAR019 | 4/27/2005 | 0.369 | 90.0 |
| 412LAR025 | 4/27/2005 | 0.340 | 97.5 |
| DIEPAMH | Laboratory Control | 0.305 | 98.8 |
| 412LAR009 | 5/16/2005 | 0.281 | 92.5 |
| 412LAR020 | 5/16/2005 | 0.308 | 100.0 |
| 412LAR025 | 5/16/2005 | 0.292 | 97.5 |
| 405SGB022 | 5/17/2005 | 0.316 | 100.0 |
| DIEPAMH | Laboratory Control | 0.269 | 97.0 |
| 405SGB025 | 5/17/2005 | 0.243 | 98.0 |
| 405SGB004 | 5/18/2005 | 0.312 | 98.0 |
| 405SGB018 | 5/18/2005 | 0.262 | 98.0 |
| DIEPAMH | Laboratory Control | 0.272 | 95.0 |
| 412LAR015 | 5/23/2005 | 0.278 | 97.5 |
| 412LAR018 | 5/23/2005 | 0.275 | 97.5 |
| 405SGB027 | 5/24/2005 | 0.278 | 97.5 |
| 801SGB017 | 5/24/2005 | 0.316 | 100.0 |
| 405SGB015 pH shift | 5/24/2005 | 0.286 | 97.5 |
| 412LAR023 pH shift | 5/24/2005 | 0.258 | 92.5 |
| DIEPAMH | Laboratory Control | 0.243 | 91.3 |
| DIEPAMH | Adj. 3500 umhos | 0.252 | 97.5 |
| 845SGB007 | 5/25/2005 | 0.229 | 80.0 |
| 405SGB011 | 5/25/2005 | 0.261 | 95.0 |
| 405SGB036 | 5/25/2005 | 0.266 | 80.0 |
| DIEPAMH | Laboratory Control | 0.326 | 100.0 |
| 405SGB034 | 6/7/2005 | 0.332 | 97.5 |
| 405SGB041 | 6/7/2005 | 0.358 | 97.5 |
| DIEPAMH | Laboratory Control | 0.217 | 95.0 |
| 405SGB010 | 6/8/2005 | 0.257 | 100.0 |
| 405SGBMRS | 6/9/2005 | 0.211 | 97.5 |
| DIEPAMH | Laboratory Control | 0.024 | 92.5 |
| 412LARVGO | 6/13/2005 | 0.275 | 95.0 |
| 412LARBBK | 6/13/2005 | 0.224 | 95.0 |
| 405SGB003 | 6/14/2005 | 0.215 | 80.0 |
| DIEPAMH | Laboratory Control | 0.326 | 96.3 |
| 405SGBWFK | 6/15/2005 | 0.336 | 92.5 |
| 405SGBNFK | 6/15/2005 | 0.347 | 90.0 |
| 405SGBEFK | 6/15/2005 | 0.301 | 83.4 |
| DIEPAMH | Laboratory Control | 0.327 | 97.5 |
| 400FDQ016 | 6/15/2005 | 0.034 | 95.5 |
| 405SGB006 | 6/16/2005 | 0.304 | 90.0 |

Table 16. Results of *H. azteca* toxicity tests conducted on samples collected for Region 4 on April 25, 2005.

| Sample ID | Sampling Date | Survival (%) |
|---|---|---|
| DIEPAMHR | Laboratory Control | 100.0 |
| 405SGB036 | 5/25/2005 | 93.8 |

***Water Chemistry***

Detailed water quality data is presented in Appendix A. Six samples had pH levels above 9 (412LAR024, 412LAR025, 405SGB025, 405SGB015, 412LAR023, and 405SGB041), which exceeded the test organisms' physiological range. The first high pH sample collected on April 26, 2005, was adjusted to below 9 for both the *C. dubia* and *P. promelas* tests. The five subsequent high pH samples were tested adjusted below 9 in the *C. dubia* and *P. promelas* tests, and also with their pH unadjusted in the *C. dubia* tests.

**Region 7**

***Ceriodaphnia dubia***

The results of the *C. dubia* chronic toxicity tests conducted on samples collected between May 9, 2005 and May 11, 2005 are summarized in Table 17. There were no statistically significant reductions in *C. dubia* survival or reproduction in any of the samples.

***Pimephales promelas***

The results of the *P. promelas* chronic toxicity tests conducted on samples collected between May 9, 2005 and May 11, 2005 are summarized in Table 18. There were no statistically significant reductions in biomass or survival in any of the tests.

Table 17. Summary of 7-day *C. dubia* toxicity test conducted on samples collected for Region 7 on May 10-11, 2005.

| Sample ID | Sampling Date | Survival (%) | Reproduction (neonates/female) |
|---|---|---|---|
| SSEPAMH | Lab Control | 90 | 25.7 |
| 713CRNVBD | 5/10/2005 | 100 | 21.0 |
| 719CVSCOT | 5/10/2005 | 100 | 27.7 |
| SSEPAMH | Lab Control | 100 | 28.0 |
| 715CRIDG1 | 5/11/2005 | 100 | 23.1 |
| 715CPVLG1 | 5/10/2005 | 100 | 25.0 |
| 715CPVOD2 | 5/10/2005 | 100 | 24.1 |

Table 18. Summary of chronic *P. promelas* toxicity tests conducted on samples collected for Region 7 on May 9-11, 2005.

| Sample ID | Sampling Date | Biomass (mg/individual) | Survival (%) |
|---|---|---|---|
| DIEPAMH | Laboratory Control | 0.484 | 97.5 |
| 723ARGRB1 | 5/9/2005 | 0.521 | 97.5 |
| 723ARINTL | 5/9/2005 | 0.500 | 95.0 |
| 700FDQ011 | 5/9/2005 | 0.532 | 100.0 |
| DIEPAMH | Laboratory Control | 0.371 | 97.5 |
| 713CRNVBD | 5/10/2005 | 0.446 | 100.0 |
| 719CVSCOT | 5/10/2005 | 0.513 | 100.0 |
| 715CRIDG1 | 5/11/2005 | 0.396 | 97.5 |
| 715CPVLG1 | 5/10/2005 | 0.422 | 92.5 |
| 715CPVOD2 | 5/10/2005 | 0.364 | 80.0 |
| DIEPAMH | Laboratory Control | 0.316 | 100.0 |
| 715CPVOD2** | 5/10/2005 | 0.291 | 85.0 |

** pathogen related toxicity

Sample 715CPVOD2, collected on May 10, 2005, exhibited Pathogen Related 'Toxicity' (PRT) in the initial test setup on May 12, 2005. Survival in the sample water was 80%. A

second test was setup on May 21, 2005, designed to reduce interference from PRT. The test employed 20 replicate one-ounce plastic cups, each containing 20 ml of sample and two organisms. All other aspects of the testing method were identical to the *P. promelas* chronic method outlined in the Methods and Materials section. Survival in the sample was 85%, and no PRT was exhibited. Results are summarized in Table 14 in the *Pimephales promelas* Data Appendix.

***Hyalella azteca***

The results of the *H. azteca* chronic toxicity test conducted on samples collected on May 9, 2005 and May 10, 2005 are summarized in Table 19. Two samples exhibited statistically significant reductions in survival: 723NROTWM and 723NRBDRY. A TIE was conducted on sample 723NRBDRY.

Table 19. Results of *H. azteca* toxicity tests conducted on samples collected for Region 7 on May 9-10, 2005.

| Sample ID | Sampling Date | Survival (%) |
|---|---|---|
| DIEPAMHR | Adj. 3000 umhos | 100.0 |
| 723ARGRB1 | 5/9/2005 | 96.0 |
| 723ARINTL | 5/9/2005 | 100.0 |
| 723NROTWM | 5/10/2005 | 71* |
| 723NRBDRY | 5/10/2005 | 0* |
| 700FDQ011 | 5/10/2005 | 95.0 |

* significantly different from control ($p<0.05$)

***TIE***

A TIE was conducted on sample 723NRBDRY, collected May 9, 2005, after the *H. azteca* exhibited 82% mortality to within 24 hours. The results of the TIE are shown in Table 20. Addition of carboxylesterase significantly and considerably improved survival of the test organisms (97% survival), and PBO increased toxicity, which suggests toxicity was potentially due to a pyrethroid insecticide. Air stripping also alleviated sample toxicity,

which may indicate that chemicals such as surfactants (often present in pesticide formulations), chlorine and/or ammonia contributed to the toxicity of the water sample.

Table 20. Survival of *Hyalella azteca* in a Toxicity Identification Evaluation of an ambient water column sample collected in Region 7 at site 723NRBDRY by Moss Landing Marine Laboratories on 5/09/05.[1,2]

| Treatment | % Survival[3] | | | |
|---|---|---|---|---|
| | Day 1 | Day 2 | Day 3 | Day 4 |
| 3000µS DIEPAMHR | 97 | 97 | 97 | 93 |
| 3000µS DIEPAMHR (HA) @ 960mg/L | 100 | 100 | 100 | 97 |
| 3000µS DIEPAMHR (HA) + MeOH @ 0.5% | 100 | 100 | 100 | 97 |
| 3000µS DIEPAMHR (HA) + eluate addback @ 3x | 0* | 0* | 0* | 0* |
| 3000µS DIEPAMHR (HA) + 960 mg/L EDTA | 97 | 83 | 80 | 80 |
| 3000µS DIEPAMHR (HA) + 480 mg/L EDTA | 97 | 93 | 90 | 90 |
| 3000µS DIEPAMHR (HA) + 1920 mg/L STS | 100 | 93 | 89 | 78 |
| 3000µS DIEPAMHR (HA) + 960 mg/L STS | 100 | 93 | 93 | 88 |
| 3000µS DIEPAMHR (HA) + 100ppb PBO | 100 | 100 | 97 | 90 |
| 3000µS DIEPAMHR (HA) + 50ppb PBO | 100 | 83 | 83 | 79 |
| 3000µS DIEPAMHR (HA) + 500X esterase | 100 | 93 | 93 | 93 |
| 3000µS DIEPAMHR (HA) air stripped | 97 | 97 | 97 | 97 |
| 3000µS DIEPAMHR C8 Blank | 100 | 100 | 97 | 97 |
| 723NRBDRY | 13* | 0* | 0* | 0* |
| 723NRBDRY + 960 mg/L EDTA | 7* | 0* | 0* | 0* |
| 723NRBDRY + 480 mg/L EDTA | 3* | 0* | 0* | 0* |
| 723NRBDRY + 240 mg/L EDTA | 10* | 3* | 0* | 0* |
| 723NRBDRY + 1920 mg/L STS | 0* | 0* | 0* | 0* |
| 723NRBDRY + 960 mg/L STS | 0* | 0* | 0* | 0* |
| 723NRBDRY + 480 mg/L STS | 0* | 0* | 0* | 0* |
| 723NRBDRY + 100ppb PBO | 0* | 0* | 0* | 0* |
| 723NRBDRY + 50ppb PBO | 0* | 0* | 0* | 0* |
| 723NRBDRY + 500X esterase | 100 | 97 | 97 | 97 |
| 723NRBDRY air stripped | 83 | 27* | 13* | 3* |
| 723NRBDRY C8 Rinsate | 83 | 43* | 27* | 23* |

1) Sample was treated through Varian C8 column on 5/13/05.

2) Test was set up on 5/15/05.

3) * indicates less than 50% survival of test organisms.

**Region 9**

The results of the *C. dubia* chronic toxicity tests conducted on samples collected between April 18, 2005 and June 2, 2005 are summarized in Table 21. Twelve samples exhibited statistically significant reduction ($p<0.05$) in reproduction. These were: 903SLSLR8, 907SSDR15, 907SDLCO2, 907SDCHC3, 907SDSVC4, 903SLGRD2, 903SLKYS3, 991TTJR05, 911TLAP04, 908SLAW02, 911TCWD10, and 911TTEC02. One sample caused a significant increase in mortality as compared to the control: 911TTEC02.

Table 21. Summary of 7-day *C. dubia* toxicity tests conducted on samples collected for Region 9 between April 18 and June 2, 2005.

| Sample ID | Sampling Date | Survival (%) | Reproduction (neonates/female) |
|---|---|---|---|
| SSEPAMH | Lab Control | 100 | 20.7 |
| 903SLSLR8 | 4/18/2005 | 100 | 12.1* |
| 907SSDR15 | 4/19/2005 | 100 | 10.5* |
| 907SDLCO2 | 4/19/2005 | 100 | 8.7* |
| 907SDCHC3 | 4/19/2005 | 90 | 3.4* |
| 907SDSVC4 | 4/19/2005 | 100 | 12.1* |
| 907SDBOC2 | 4/19/2005 | 100 | 26.4 |
| SSEPAMH | Lab Control | 100 | 19.1 |
| 903SLGRD2 | 4/20/2005 | 100 | 7.8* |
| 903SLIRS2 | 4/20/2005 | 100 | 17.9 |
| 903SLSLR2 | 4/20/2005 | 90 | 18.7 |
| 903SLMSA2 | 4/20/2005 | 100 | 16.2 |
| 903SLKYS3 | 4/20/2005 | 100 | 6.0* |
| SSEPAMH | Lab Control | 100 | 21.5 |
| 991TTJR05 | 5/31/2005 | 60 | 8.8* |
| 909SSWR03 | 6/1/2005 | 90 | 16.8 |
| 911TLAP04 | 6/1/2005 | 90 | 4.6* |
| 908SLAW02 | 6/1/2005 | 100 | 13.4* |
| 900FDQ014 | 6/1/2005 | 100 | 7.4* |
| SSEPAMH | Lab Control | 100 | 20.7 |
| 911TCWD10 | 6/2/2005 | 3 | 12.8* |
| 911TTEC02 | 6/2/2005 | 0 | 0* |
| SSEPAMH | Lab Control | 100 | NAP** |
| 911TTEC02 | 6/2/2005 | 0* | NAP** |
| 911TTEC02 pH adjusted to 7.3 | 6/2/2005 | 0* | NAP** |
| 911TTEC02 pH adjusted to 6.3 | 6/2/2005 | 0* | NAP** |

***TIE***

An acute graduated pH shift test was setup with Region 9 sample 911TTECO2 collected June 2, 2005, because it contained 22 mg/L ammonia. Ammonia levels that exceed 5mg/L are considered toxic to the test organisms. At lower pH levels ammonia ($NH_3$) is converted to ionic ammonium ($NH_4^+$), which is less toxic to aquatic organisms. In the graduated pH shift test, the sample was set up with its pH of 8.3 unaltered, with the sample's pH lowered to 7.3, and with the sample's pH lowered to 6.3. All three of the treatments exhibited 100% mortality in the test (Table 20). Upon test termination, the ammonia concentration was measured in the three treatments and the control. Unadjusted 911TTEC02 had an ammonia level exceeding 20 mg/L, but the two pH adjusted treatments and the control had ammonia levels below 2mg/L. The persistence of toxicity at low pH suggests toxicity was due, in part, to other toxicant(s) besides ammonia.

***Selenastrum capricornutum***

The results of the *S. capricornutum* chronic toxicity tests conducted on samples collected between April 18, 2005 and June 2, 2005 are summarized in Table 22. Five samples exhibited a statistically significant reduction in cell count as compared to the control: 907SDFRC2, 903SLGRD2, 903SLKYS3, 909SSWR08, and 911TTEC02.

Water samples from Region 9 often exceed an EC of 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 test was therefore setup to determine how high ECs would affect cell count. The EC of glass distilled (GD) water was adjusted 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 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.

Table 22. Summary of 3rd quarter 96-hour *S. capricornutum* chronic toxicity test results for Region 9.

| Sample ID | Sampling Date | Average Cell Count (x $10^6$) | % CV |
|---|---|---|---|
| Glass Distilled | Laboratory Control | 1.800 | 4.9 |
| Glass Distilled | Adj. 2000 umhos | 1.734 | 9.4 |
| Glass Distilled | Adj. 3000 umhos | 1.698 | 6.5 |
| 903SLSLR8[a] | 4/18/2005 | 1.861 | 6.4 |
| 907SSDR15[a] | 4/19/2005 | 1.511 | 10.3 |
| 907SDALV3[b] | 4/19/2005 | 2.035 | 6.8 |
| 907SDFRC2[b] | 4/19/2005 | 1.291* | 2.9 |
| 907SDLCO2[a] | 4/19/2005 | 1.397 | 11.6 |
| 907SDCHC3[a] | 4/19/2005 | 2.231 | 5.2 |
| 907SDSVC4 | 4/19/2005 | 1.895 | 14.7 |
| 907SDBOC2 | 4/19/2005 | 1.891 | 15.3 |
| Glass Distilled | Laboratory Control | 1.922 | 5.4 |
| Glass Distilled | Adj. 2000 umhos | 1.740 | 10.3 |
| 903SDCHC3 | 4/19/2005 | 2.009 | 12.8 |
| Glass Distilled | Laboratory Control | 3.120 | 5.3 |
| Glass Distilled | Adj. 2000 umhos | 3.129 | 5.6 |
| Glass Distilled | Adj. 3000 umhos | 2.848 | 8.0 |
| 903SLGRD2[a] | 4/20/2005 | 2.544* | 5.9 |
| 903SLIRS2 | 4/20/2005 | 5.142 | 5.5 |
| 903SLSLR2 | 4/20/2005 | 5.255 | 2.9 |
| 903SLMSA2[a] | 4/20/2005 | 3.613 | 2.1 |
| 903SLKYS3[a] | 4/20/2005 | 2.164 | 11.8 |
| Glass Distilled | Laboratory Control | 1.369 | 15.9 |
| Glass Distilled | Adj. 5000 umhos | 1.153 | 7.3 |
| Glass Distilled | Adj. 7000 umhos | 0.964 | 3.3 |
| 908PPAR04 | 5/31/2005 | 1.441 | 7.9 |
| 911TTJR05 | 5/31/2005 | 1.571 | 4.4 |
| 908PTEL02[c] | 5/31/2005 | 1.906 | 10.7 |
| 909SSWR08[d] | 5/31/2005 | 0.499* | 2.6 |
| 909SSWR03 | 6/1/2005 | 1.515 | 15.7 |
| 911TLAP04 | 6/1/2005 | 1.889 | 6.9 |
| 908SLAW02 | 6/1/2005 | 1.572 | 13 |
| 900FDQ014 | 6/1/2005 | 1.911 | 6.7 |
| Glass Distilled | Laboratory Control | 1.285 | 9.1 |
| 911TCWD10 | 6/2/2005 | 1.498 | 9.1 |
| 911TTEC02 | 6/2/2005 | 0.126* | 8.8 |

* Significantly different from control ($p<0.05$)

a: Compared to the 2000 umhos control

b: Compared to the 3000 umhos control

c: Compared to the 5000 umhos control

d: Compared to the 7000 umhos control

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 the appropriate EC using seawater. Seawater is used to adjust the GD water because it is natural water that may better represent constituents contributing to the high electrical conductivities in the sample waters than NaCl. However, it is understood that ionic ratios in various surface waters differ and are not likely to be similar to 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 screening ambient samples.

***Hyalella azteca***

The results of the *H. azteca* chronic toxicity tests conducted on samples collected on April 19, and May 31, 2005 are summarized in Table 23. There were no statistically significant reductions in survival in the tests.

Table 23. Results of *H. azteca* toxicity tests conducted on samples collected for Region 9 on April 19, 2005 and May 31, 2005.

| Sample ID | Sampling Date | Survival (%) |
|---|---|---|
| DIEPAMHR | Adj. 3000 umhos | 100.0 |
| 907SDALV3 | 4/19/2005 | 98.0 |
| 907SDFRC2 | 4/19/2005 | 98.0 |
| DIEPAMHR | Laboratory Control | 80.9 |
| 908PPAR04 | 5/31/2005 | 96.0 |
| 908PTEL02 | 5/31/2005 | 90.8 |
| 909SSWR08 | 5/31/2005 | 100.0 |

* significantly different from control ($p<0.05$)

***Water Chemistry***

Detailed water quality data is presented in Appendix A. Prior to *C. dubia, S. capricornutum, P. promelas,* and *H. azteca* test initiation and at test termination, 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.

## July 1-September 30, 2005

### Region 4

***Ceriodaphnia dubia***

The results of the *C. dubia* chronic toxicity tests conducted on samples collected between June 16, 2005 and July 12, 2005 are summarized in Table 24. Seventeen samples exhibited a statistically significant reduction ($p<0.05$) in reproduction as compared to the control: 405SGB006, 412LARBLL, 412LARBLL pH shifted, 412LAR013, 412LARTJA, 412LARTJA pH shifted, 412LARSCO, 412LAR031, 412LAR031 pH shifted, 412LAR007, 412LARRHO (collected June 29, 2005), 412LARRHO (collected June 29, 2005) pH shifted, 412LARBLL pH shifted, 405SGBNFK, 412LAR024, 412LAR024 pH shifted, and 412LARRHO (collected July 12, 205).

Eight samples caused a significant increase in mortality as compared to the control: 405SGB006, 412LARBLL, 412LARBLL pH shifted, 412LARRHO (collected June 29, 2005), 412LARRHO pH shifted (collected June 29, 2005), 412LAR024, 412LAR024 pH shifted, and 412LARRHO (collected July 12, 2005).

The results of the *C. dubia* chronic toxicity tests conducted on the samples collected by Southern California Coastal Water Research Project (SCCWRP) between June 17, 2005 and June 30, 2005 are summarized in Tables B1-3 of the SCCWRP *C. dubia* section of the Data Appendix. Two samples exhibited a statistically significant reduction ($p<0.05$) in reproduction as compared to the control: SGUR-1 and SGUR-2.

Table 24. Summary of 7-day *C. dubia* toxicity tests conducted on samples collected for Region 4 between June 15 and July 13, 2005.

| Sample ID | Sampling Date | Survival (%) | Reproduction (neonates/female) |
|---|---|---|---|
| SSEPAMH | Lab Control | 100 | 24.0 |
| 405SGB006 | 6/16/2005 | 60* | 2.5* |
| SSEPAMH | Lab Control | 100 | 23.1 |
| 405SGBCLD | 6/17/2005 | 100 | 22.2 |
| SSEPAMH | Lab Control | 100 | 24.3 |
| 412LARBLL | 6/20/2005 | 0* | 0* |
| 412LARBLL pH shift | 6/20/2005 | 10* | 0* |
| 412LAR013 | 6/21/2005 | 100 | 15.7* |
| 412LARTJA | 6/21/2005 | 80 | 7.8* |
| 412LARTJA pH shift | 6/21/2005 | 90 | 0* |
| SSEPAMH | Lab Control | 88.9 | 21.3 |
| 405SGB042 | 6/24/2005 | 100 | 16.4 |
| SSEPAMH | Lab Control | 100 | 17.2 |
| 412LARVGO | 6/27/2005 | 25.3 | 100.0 |
| SSEPAMH | Lab Control | 100 | 20.8 |
| 412LARSCO | 6/28/2005 | 100 | 11.4 |
| 412LAR031 | 6/28/2005 | 100 | 3.7* |
| 412LAR031 pH shift | 6/28/2005 | 90 | 0* |
| 412LAR007 | 6/29/2005 | 80 | 15.4 |
| 412LAR007 pH shift | 6/29/2005 | 80 | 24.7 |
| 412LARRHO | 6/29/2005 | 0* | 0* |
| 412LARRHO pH shift | 6/29/2005 | 0* | 0* |
| SSEPAMH | Lab Control | 95 | 21.2 |
| 405SGB065 | 6/29/2005 | 100 | 23.5 |
| SGUR-01 | 6/30/2005 | 90 | 11.6* |
| SGUR-02 | 6/30/2005 | 100 | 13.9* |
| 412LARCMP | 6/30/2005 | 80 | 23.7 |
| SSEPAMH | Lab Control | 100 | 21.1 |
| 412LAR024 | 7/11/2005 | 0* | 0* |
| 412LARBLL | 7/11/2005 | 100 | 19.7 |
| 412LAR024 pH shift | 7/11/2005 | 0* | 0* |
| 412LARBLL pH shift | 7/11/2005 | 100 | 8.8* |
| 412LARRHO | 7/11/2005 | 0* | 0* |
| 405SGBNFK | 7/12/2005 | 90 | 7.0* |

* significantly different from control ($p<0.05$)

***Pimephales promelas***

The results of the *P. promelas* chronic toxicity tests conducted on samples collected between June 16, 2005 and July 12, 2005 are summarized in Table 25. One sample, 412LARRHO, caused statistically significant ($p<0.05$) reductions in biomass and survival as compared to the control.

Table 25. Summary of 7-day *P. promelas* chronic toxicity test results for samples collected for Region 4 between June 15 and July 13, 2005.

| Sample ID | Sampling Date | Biomass (mg/individual) | Survival (%) |
|---|---|---|---|
| DIEPAMH | Laboratory Control | 0.327 | 97.5 |
| 405SGB006 | 6/16/2005 | 0.327 | 97.5 |
| DIEPAMH | Laboratory Control | 0.419 | 97.5 |
| 412LARBLL pH adjusted | 6/20/2005 | 0.413 | 92.5 |
| 412LAR016 | 6/20/2005 | 0.491 | 90.0 |
| 412LAR013 | 6/21/2005 | 0.387 | 97.5 |
| 412LARTJA pH adjusted | 6/21/2005 | 0.416 | 97.5 |
| DIEPAMH | Laboratory Control | 0.390 | 97.5 |
| 412LARSCO | 6/28/2005 | 0.382 | 97.5 |
| 412LAR031 | 6/28/2005 | 0.421 | 92.5 |
| DIEPAMH | Laboratory Control | 0.411 | 100.0 |
| 412LARVGO | 6/27/2005 | 0.460 | 97.5 |
| 412LAR007 | 6/29/2005 | 0.451 | 97.5 |
| 412LARRHO | 6/29/2005 | 0.499 | 97.5 |
| DIEPAMH | Laboratory Control | 0.415 | 100.0 |
| 412LARCMP | 6/30/2005 | 0.405 | 100.0 |
| DIEPAMH | Laboratory Control | 0.433 | 100.0 |
| 412LAR024, pH shift | 7/11/2005 | 0.492 | 100.0 |
| 412LARBLL, pH adjusted | 7/11/2005 | 0.508 | 100.0 |
| 412LARRHO | 7/11/2005 | 0* | 0* |
| DIEPAMH | Laboratory Control | 0.508 | 100.0 |
| 405SGBNFK | 7/12/2005 | 0.492 | 98.0 |

*Significantly different from control ($p<0.05$)

***Water Chemistry***

Detailed water quality data is presented in Appendix A. Four samples had pH levels above 9 (412LARBLL and 412LARTJA collected on June 20, 2005; 412LAR024, and 412LARBLL collected on July 11, 2005), which exceeded the test organisms' physiological range. The high pH samples were tested adjusted below 9 in the *C. dubia* and *P. promelas* tests, and also with their pH unadjusted in the *C. dubia* tests.

***TIEs***

Three Phase I TIEs and two dilution series were conducted on toxic samples:

Sample 412LARRHO, collected July 11, 2005, caused 100% mortality within 24 hours to both *C. dubia* and *P. promelas* (Tables 26, 27). A dilution series for both species was setup. The *C. dubia* exhibited 100% mortality within 48 hours in all of the sample dilutions, which indicated there were greater than 16 toxic units (TUs) in the sample. The *P. promelas* exhibited 100% mortality in the 100, 50 and 25% dilutions, which suggested that 8 TUs were present in the sample.

Phase I TIEs with *P. promelas* and *C. dubia* demonstrated that sodium thiosulfate eliminated the acute toxicity (Tables 28, 29) for both species. This indicates that (a) cationic metal(s) caused at significant part of the observed toxicity. In the *C. dubia* TIE the carbon column eluate also caused 100% mortality within 72 hours when added to control water, but the fathead minnows survived in this treatment. This suggests that a nonpolar compound was present at a concentration toxic to *C. dubia*, but not to *P. promelas*, and contributed to the sample's toxicity to *C. dubia.* Air stripping alleviated *C. dubia* toxicity to some degree, indicating that a volatile toxic compound such as surfactants (often present in pesticide formulations), chlorine and/or ammonia was also present.

Table 26. Summary of 96-hour *C. dubia* dilution series conducted on sample 412LARRHO collected for Region 4 on 7/11/05.[2]

| Treatment | Survival (%)[1] | | Final pH |
|---|---|---|---|
| | 24 hrs | 48 hrs | at 24 hrs |
| Laboratory Control | 100 | 100 | 7.97 |
| 412LARRHO (6.25%) | 0* | 0* | 7.83 |
| 412LARRHO (12.5%) | 0* | 0* | 7.94 |
| 412LARRHO (25%) | 0* | 0* | 7.96 |
| 412LARRHO (50%) | 0* | 0* | 8.03 |
| 412LARRHO (100%) | 0* | 0* | 8.13 |

1. * indicates a survival rate below 50%.
2. This test was set up on 7/14/05.
   The test was terminated after 48 hours due to the death of all animals in the ambient water treatments.
   The death of the animals in the 6.25% treatment indicates the presence of greater than 16 Toxic Units in the sample.

Table 27. Summary of 96-hour *P. promelas* dilution series conducted on sample 412LARRHO collected for Region 4 on 7/11/05.[2]

| Treatment | Survival (%)[1] | | | | Final pH |
|---|---|---|---|---|---|
| | 24 hrs | 48 hrs | 72 hrs | 96 hrs | at 24 hrs |
| Laboratory Control | 100 | 100 | 100 | 95 | 7.99 |
| 412LARRHO (6.25%) | 100 | 100 | 100 | 100 | 7.96 |
| 412LARRHO (12.5%) | 100 | 95 | 80 | 80 | 8.00 |
| 412LARRHO (25%) | 15* | 10* | 0* | 0* | 8.11 |
| 412LARRHO (50%) | 0* | 0* | 0* | 0* | 8.08 |
| 412LARRHO (100%) | 0* | 0* | 0* | 0* | 8.20 |

1. * indicates a survival rate below 50%.
2. This test was set up on 7/14/05.

Table 28. Summary of 96-hour *C. dubia* toxicity identification evaluation conducted on sample 412LARRHO collected by Moss Landing Marine Laboratories for SWAMP Region 4 on 7/11/05.[2]

| Treatment | Survival (%)[1] | | | | Final pH |
|---|---|---|---|---|---|
| | 24 hrs | 48 hrs | 72 hrs | 96 hrs | at 24 hrs |
| Laboratory Control | 100 | 100 | 100 | 100 | 7.83 |
| SSEPAMH (HA) @ 168 mg/L | 100 | 100 | 100 | 100 | 8.17 |
| SSEPAMH (HA) + MeOH @ 0.5% | 100 | 100 | 100 | 100 | 8.17 |
| SSEPAMH (HA) + eluate addback @ 3x | 100 | 100 | 0* | 0* | 7.80 |
| SSEPAMH (HA) + 168 mg/L EDTA | 100 | 100 | 95 | 95 | 8.18 |
| SSEPAMH (HA) + 84 mg/L EDTA | 100 | 100 | 100 | 100 | 7.98 |
| SSEPAMH (HA) + 336 mg/L STS | 100 | 100 | 85 | 75 | 8.15 |
| SSEPAMH (HA) + 168 mg/L STS | 100 | 100 | 100 | 95 | 8.17 |
| SSEPAMH (HA) + 100 ppb PBO | 100 | 100 | 100 | 100 | 8.18 |
| SSEPAMH (HA) + 100x esterase | 100 | 100 | 100 | 100 | 7.94 |
| SSEPAMH (HA) air stripped | 100 | 100 | 100 | 100 | 8.18 |
| SSEPAMH C8 blank | 100 | 100 | 100 | 100 | 7.84 |
| 412LARRHO | 0* | 0* | 0* | 0* | 8.14 |
| 412LARRHO + 168 mg/L EDTA | 0* | 0* | 0* | 0* | 7.80 |
| 412LARRHO + 84 mg/L EDTA | 0* | 0* | 0* | 0* | 8.00 |
| 412LARRHO + 42 mg/L EDTA | 0* | 0* | 0* | 0* | 8.07 |
| 412LARRHO + 336 mg/L STS | 95 | 95 | 95 | 95 | 8.01 |
| 412LARRHO + 168 mg/L STS | 100 | 100 | 100 | 100 | 7.96 |
| 412LARRHO + 84 mg/L STS | 100 | 100 | 100 | 100 | 8.00 |
| 412LARRHO + 100 ppb PBO | 0* | 0* | 0* | 0* | 8.12 |
| 412LARRHO + 100x esterase | 0* | 0* | 0* | 0* | 8.14 |
| 412LARRHO air stripped | 100 | 0* | 0* | 0* | 7.86 |
| 412LARRHO C8 rinsate | 0* | 0* | 0* | 0* | 8.16 |

1. * indicates a survival rate below 50%.
2. The sample was processed through C8 columns on 7/14/05, and this test was set up on 7/15/05.

Table 29. Summary of 96-hour *P. promelas* toxicity identification evaluation conducted on sample 412LARRHO collected by Moss Landing Marine Laboratories for SWAMP Region 4 on 7/11/05.[2]

| Treatment | Survival (%)[1] | | | | Final pH |
|---|---|---|---|---|---|
| | 24 hrs | 48 hrs | 72 hrs | 96 hrs | at 24 hrs |
| Laboratory Control | 100 | 100 | 95 | 85 | 7.97 |
| DIEPAMH (HA) @ 168 mg/L | 100 | 100 | 100 | 95 | 8.25 |
| DIEPAMH (HA) + MeOH @ 0.5% | 100 | 100 | 95 | 90 | 8.23 |
| DIEPAMH (HA) + eluate addback @ 3x | 100 | 100 | 100 | 95 | 8.23 |
| DIEPAMH (HA) + 336 mg/L EDTA | 100 | 100 | 100 | 85 | 6.57 |
| DIEPAMH (HA) + 168 mg/L EDTA | 100 | 100 | 100 | 100 | 6.57 |
| DIEPAMH (HA) + 1008 mg/L STS | 100 | 100 | 100 | 95 | 8.23 |
| DIEPAMH (HA) + 504 mg/L STS | 100 | 100 | 100 | 95 | 8.21 |
| DIEPAMH (HA) + 100x esterase | 95 | 95 | 95 | 95 | 8.03 |
| DIEPAMH (HA) air stripped | 100 | 100 | 100 | 100 | 8.24 |
| DIEPAMH C8 blank | 100 | 100 | 100 | 100 | 7.99 |
| 412LARRHO | 0* | 0* | 0* | 0* | 8.06 |
| 412LARRHO + 336 mg/L EDTA | 0* | 0* | 0* | 0* | 4.98 |
| 412LARRHO + 168 mg/L EDTA | 0* | 0* | 0* | 0* | 7.62 |
| 412LARRHO + 84 mg/L EDTA | 0* | 0* | 0* | 0* | 7.91 |
| 412LARRHO + 1008 mg/L STS | 100 | 100 | 95 | 84 | 8.05 |
| 412LARRHO + 504 mg/L STS | 100 | 100 | 100 | 100 | 8.06 |
| 412LARRHO + 252 mg/L STS | 100 | 100 | 100 | 100 | 8.05 |
| 412LARRHO + 100x esterase | 0* | 0* | 0* | 0* | 8.11 |
| 412LARRHO air stripped | 100 | 100 | 0* | 0* | 8.00 |
| 412LARRHO C8 rinsate | 0* | 0* | 0* | 0* | 8.12 |

1. * indicates a survival rate below 50%.
2. The sample was processed through C8 columns on 7/14/05, and this test was set up on 7/15/05.

Sample 412LAR024, collected July 11, 2005 caused 100% mortality within 96 hours to *C. dubia.* The column rinsate was the only treatment that completely removed the acute toxicity in the phase I TIE (Table 30), which suggests that (a) nonpolar compound(s) caused the toxicity. The addition of carboxylesterase slowed mortality, which suggests a pyrethroid as a possible contributor to sample toxicity. However, the addition of carboxylesterase did not significantly improve survival as compared to the ambient sample by the end of the test. In conclusion, the toxicity in this sample was likely due to a mixture of hydrophobic, organic contaminants.

Table 30. Summary of 96-hour *C. dubia* toxicity identification evaluation conducted on sample 412LAR024 collected for Region 4 on 7/11/05.[2]

| Treatment | Survival (%)[1] | | | | Final pH |
|---|---|---|---|---|---|
| | 24 hrs | 48 hrs | 72 hrs | 96 hrs | at 24 hrs |
| Laboratory Control | 100 | 100 | 100 | 100 | 7.96 |
| SSEPAMH (HA) @ 276 mg/L | 100 | 100 | 100 | 100 | 8.32 |
| SSEPAMH (HA) + MeOH @ 0.5% | 100 | 100 | 100 | 100 | 8.36 |
| SSEPAMH (HA) + eluate addback @ 3x | 40* | 0* | 0* | 0* | 8.33 |
| SSEPAMH (HA) + 276 mg/L EDTA | 100 | 100 | 100 | 100 | 7.69 |
| SSEPAMH (HA) + 138 mg/L EDTA | 100 | 100 | 100 | 100 | 8.12 |
| SSEPAMH (HA) + 552 mg/L STS | 100 | 85 | 75 | 70 | 8.30 |
| SSEPAMH (HA) + 276 mg/L STS | 100 | 100 | 100 | 100 | 8.31 |
| SSEPAMH (HA) + 100 ppb PBO | 100 | 80 | 65 | 65 | 8.34 |
| SSEPAMH (HA) + 100x esterase | 100 | 95 | 95 | 90 | 8.23 |
| SSEPAMH (HA) air stripped | 100 | 95 | 95 | 95 | 8.39 |
| SSEPAMH C8 blank | 100 | 95 | 95 | 95 | 7.84 |
| 412LAR024 | 90 | 40* | 30* | 20* | 8.91 |
| 412LAR024 + 276 mg/L EDTA | 86 | 10* | 0* | 0* | 7.89 |
| 412LAR024 + 138 mg/L EDTA | 85 | 0* | 0* | 0* | 8.44 |
| 412LAR024 + 69 mg/L EDTA | 95 | 30* | 25* | 10* | 8.74 |
| 412LAR024 + 552 mg/L STS | 100 | 26* | 5* | 5* | 8.98 |
| 412LAR024 + 276 mg/L STS | 100 | 0* | 0* | 0* | 8.86 |
| 412LAR024 + 138 mg/L STS | 100 | 5* | 0* | 0* | 8.90 |
| 412LAR024 + 100 ppb PBO | 85 | 0* | 0* | 0* | 8.83 |
| 412LAR024 + 100x esterase | 95 | 65 | 50* | 15* | 8.68 |
| 412LAR024 air stripped | 95 | 20* | 15* | 10* | 8.27 |
| 412LAR024 C8 rinsate | 100 | 100 | 100 | 90 | 8.36 |

1. * indicates a survival rate below 50%.
2. The sample was processed through C8 columns on 7/16/05, and this test was set up on 7/16/05.

## Region 9

### *Ceriodaphnia dubia*

The results of the *C. dubia* chronic toxicity test conducted on samples collected between September 6, 2005 and September 7, 2005 are summarized in Table 31. There were no statistically significant reductions in survival or reproduction in the test.

Table 31. Summary of 7-day *C. dubia* toxicity tests conducted on samples collected for Region 9 on September 6-7, 2005.

| Sample ID | Sampling Date | Survival (%) | Reproduction (neonates/female) (x) | (se) |
|---|---|---|---|---|
| SSEPAMH | Laboratory Control | 95 | 25.2 | 6.07 |
| 908PPAR04 | 9/6/2005 | 80 | 18.0 | 11.95 |
| 909SSWR03 | 9/7/2005 | 80 | 32.8 | 2.27 |
| 911TLAP04 | 9/7/2005 | 100 | 30.4 | 2.62 |

* significantly different from control ($p<0.05$)

### *Selenastrum capricornutum*

The results of the *S. capricornutum* chronic toxicity test conducted on samples collected between September 6, 2005 and September 7, 2005 are summarized in Table 32. Two samples exhibited statistically significant reduction ($p<0.05$) in cell count as compared to the control: 909SSWR08 and 911TTEC02.

UCD ATL includes high EC controls if there are water samples with EC >1500 µmhos. The high EC control water consists of GD water adjusted to the appropriate EC using seawater. Seawater is used to adjust the GD water because it is natural water that may better represent constituents contributing to the high electrical conductivities in the sample waters than NaCl. However, it is understood that ionic ratios in various surface waters differ and are not likely to be similar to 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 screening ambient samples.

Table 32. Summary of 96-hour *S. capricornutum* chronic toxicity test results for samples collected for Region 9 on September 6/7, 2005.

| Sample ID | Sampling Date | Average Cell Count (x $10^6$) | % CV |
|---|---|---|---|
| Glass Distilled | Laboratory Control | 1.271 | 3.9 |
| Glass Distilled | Adj. 3000 umhos | 1.198 | 5.4 |
| Glass Distilled | Adj. 5000 umhos | 1.160 | 4.5 |
| Glass Distilled | Adj. 7000 umhos | 1.029 | 2.4 |
| 908PTEL02[a] | 9/6/2005 | 1.016 | 6.7 |
| 909SSWR08[b] | 9/6/2005 | 0.388* | 11.9 |
| 908PPAR04 | 9/6/2005 | 1.045 | 7.3 |
| 909SSWR03 | 9/7/2005 | 1.414 | 11.7 |
| 911TLAP04 | 9/7/2005 | 1.520 | 15.3 |
| 911TTEC02[c] | 9/7/2005 | 0.113* | 7.0 |

* Significantly different from control ($p<0.05$)

a: Compared to the 3000 umhos control

b: Compared to the 5000 umhos control

c: Compared to the 7000 umhos control

***Hyalella azteca***

The results of the *H. azteca* chronic toxicity test conducted on samples collected on September 6 and 7, 2005 are summarized in Table 33. One sample exhibited a statistically significant reduction ($p<0.05$) in survival: 911TTEC02.

***Water Chemistry***

Detailed water quality data is presented in Appendix A. Prior to *C. dubia, S. capricornutum, P. promelas,* and *H. azteca* test initiation and at test termination, temperature, dissolved oxygen (DO), pH, electrical conductivity (EC), alkalinity, hardness, and ammonia were measured. In the *S. capricornutum* test pH was 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.

Table 33. Summary of 10-day *H. azteca* toxicity test results for samples collected for Region 9 on September 6/7, 2005.

| Treatment | Survival (%)[1] | |
|---|---|---|
| | x | se |
| Lab Control (DIEPAMHR) | 99.0 | 0.9 |
| Lab Control (DIEPAMHR @ 7000 umhos) | 98.0 | 2.0 |
| 908PTEL02 | 98.0 | 4.5 |
| 909SSWR08 | 100.0 | 0.0 |
| 911TTEC02 | 2.0* | 4.5 |

1. Highlighted areas indicate a significant increase in mortality as compared to the laboratory control.
2. This test was set up on 9/8/05.
3. The laboratory control met the criteria for test acceptability.

* Signifcantly different from control ($p<0.05$).

## October 1-December 31, 2005

### Region 7

#### *Ceriodaphnia dubia*

The results of the *C. dubia* chronic toxicity test conducted on samples collected between October 24, 2005 and October 26, 2005 are summarized in Table 34. There were no statistically significant reductions in survival. However, all five water samples caused significant reductions in fecundity: 713CRNVBD, 715CPVLG1, 715CPVOD2, 715CRIDG1 and 719CVSC52.

#### *Pimephales promelas*

The results of the *P. promelas* chronic toxicity tests conducted on samples collected between October 24, 2005 and October 26, 2005 are summarized in Table 35. Samples from

715CPVOD2 and 719CVSC52 caused significant reductions in survival. Biomass was also reduced in fish exposed to 719CVSC52 water. The cause of toxicity at 719CVSC52 was likely due to high ammonia levels, which was confirmed by pH adjustment of the sample and subsequent alleviation of toxicity (Table 36).

***Hyalella azteca***

The results of the *H. azteca* chronic toxicity tests conducted on samples collected between October 24, 2005 and October 26, 2005 are summarized in Table 37. Water samples from sites 723NRBDRY and 723NROTWM, as well as the Quality Assurance sample 700FDQ012 caused significant reductions in amphipod survival.

Table 34. Summary of 7-day *C. dubia* toxicity tests conducted on samples collected for Region 7 on 10/24/05 - 10/26/05.[1]

| Treatment | Sampling Date | Reproduction[2,3] (neonates/adult) | | Survival[2] (%) |
|---|---|---|---|---|
| | | x | se | |
| Laboratory Control (SSEPAMH) | NAP | 28.9[P] | 1.51 | 95[P] |
| 713CRNVBD | 102405 | 15.0* | 2.23 | 100 |
| 715CPVLG1 | 102505 | 16.0* | 2.42 | 90 |
| 715CPVOD2 | 102505 | 21.8* | 0.83 | 100 |
| 715CRIDG1 | 102505 | 21.1* | 1.39 | 100 |
| Laboratory Control (SSEPAMH) | | 29[P] | 1.23 | 100[P] |
| 719CVSC52 | 102605 | 9.3* [4] | 2.72 | 30* |
| Quality Assurance Sample | | | | |
| 700CSSBB | 102505 | 27.2 | 1.70 | 90 |

P. The laboratory control met all EPA criteria for test acceptability. 95% of the daphnids had a third brood.

* indicates a significant reduction (one-tailed alpha = 0.05) in reproduction or increase in mortality relative to the laboratory control water.

1. This test was set up on 10/26/05 .
3. Ceriodaphnia reference toxicant test conducted on 10/06/05 shows that reproductive NaCl NOEC = 1027 uS/cm, LOEC = 1953 uS/cm. Therefore, reproductive toxicity observed in the ambient samples may be wholly or partially caused by high conductivities.
4. Reduction in reproduction and mortality in the ambient sample appears to be due to high ammonia levels (13 mg/L), which exceeded the 48 hr LC50.

Table 35. Summary of 7-day *P. promelas* toxicity tests conducted on samples collected for Region 7 on 10/24/05 - 10/26/05.[2]

| Treatment | Sampling Date | Biomass[1] (mg/individ.) | | Survival (%)[1] | | Final pH at 24 hours |
|---|---|---|---|---|---|---|
| | | x | se | x | se | |
| Laboratory Control | NAP | 0.420[P] | 0.018 | 100[P] | 0.0 | 7.91 |
| 713CRNVBD | 102405 | 0.403 | 0.018 | 97.5 | 2.5 | 8.19 |
| 715CPVLG1 | 102505 | 0.447 | 0.027 | 97.5 | 2.5 | 8.16 |
| 715CPVOD2 | 102505 | 0.395 | 0.038 | 72.9* | 10.7 | 8.15 |
| 715CRIDG1 | 102505 | 0.425 | 0.016 | 97.5 | 2.5 | 8.28 |
| Laboratory Control | NAP | 0.406[P] | 0.011 | 95.0[P] | 2.9 | 7.82 |
| 719CVSC52 [3] | 102605 | 0.080* | 0.036 | 20.0* | 9.1 | 8.21 |
| 723ARINTL | 102605 | 0.424 | 0.031 | 95.0 | 2.9 | 8.27 |
| Quality Assurance | | | | | | |
| 700FDHBB | 102505 | 0.446 | 0.015 | 100.0 | 0.0 | 7.87 |

P. The laboratory control met the criteria for test acceptability.

* indicates a significant decrease in survival or biomass when compared to the laboratory control. The biomass and mortality endpoints were analyzed with T-tests (one-tailed alpha = 0.05).

2. This test was set up on 10/26/05.

3. Toxicity in 719CVSC52 was most likely due to high ammonia, which exceeded the fathead minnow 96-h LC50.

Table 36. Summary of 96-hr *P. promelas* toxicity test conducted on pH-adjusted sample 719CVSC52 collected for Region 7 on 10/26/05.[2]

| Treatment | Survival (%)[1,3] x | se |
|---|---|---|
| Laboratory Control | 100[P] | 0.0 |
| 719CVSC52 | 0* | 0.0 |
| DIEPAMH @ pH = 6.0 | 95 | 5.0 |
| 719CVSC52 @ pH = 6.0 | 90 | 5.8 |
| DIEPAMH @ pH = 6.8 | 100 | 0.0 |
| 719CVSC52 @ pH = 6.8 | 95 | 5.0 |
| DIEPAMH @ pH = 7.8 | 100 | 0.0 |

P. The laboratory control met the criteria for test acceptability.

* indicates a substantial decrease in survival compared to the laboratory control.

2. This test was set up on 11/03/05.

3. Survival of fish in the pH adjusted ambient samples supports ammonia was the likely cause of toxicity.

Table 37. Summary of 10-day *H. azteca* toxicity test conducted on samples collected for Region 7 on 10/25/05 and 10/26/05.[2]

| Treatment | Survival (%)[1] x | se |
|---|---|---|
| Laboratory Control | 97[P] | 1.6 |
| Lab. Control @ 5.60 mS/cm | 98 | 2.0 |
| 723ARINTL | 96 | 2.4 |
| 723NRBDRY | 0* | 0.0 |
| 723ARGRB1 | 96 | 2.4 |
| 723NROTWM | 18* | 5.5 |
| Quality Assurance Samples | | |
| 700FDQ012 | 26* | 5.8 |

P. The laboratory control met the criteria for test acceptability.

* indicates a significant increase in mortality when compared to the laboratory control. The mortality endpoint was analyzed with T-tests (one-tailed alpha = 0.05).

2. This test was set up on 10/27/05.

***TIE***

Phase I and II TIEs were conducted on the sample from site 723NRBDRY (Tables 38, 39). Toxicity of the sample was alleviated by C8 treatment, which removes non-polar organic compounds. When added to control water, the C8 eluate caused 100% mortality in the test organisms demonstrating that most of the toxicity was due to (a) non-polar organic compound(s).

Toxicity was alleviated by the addition of carboxylesterase, which is an indication of toxicity caused by pyrethroid pesticides. Addition of the pyrethroid-synergist PBO enhanced sample toxicity, which provides additional evidence for the presence of pyrethroids at toxic concentrations. Metal chelators (EDTA, STS) or air stripping did not reduce the toxicity.

Table 38. Summary of 96-hr *H. azteca* TIE conducted on a sample from 723NRBDRY collected on 10/25/05.[2]

| Treatment | Survival (%) | | | |
|---|---|---|---|---|
| | 24 hr | 48 hr | 72 hr | 96 hr |
| DIEPAMHR[3] | 97 | 97 | 87 | 87 |
| DIEPAMHR (HA) @ 812 mg/L | 100 | 100 | 100 | 97 |
| DIEPAMHR (HA) + MeOH @ 0.5% | 100 | 100 | 87 | 53 |
| DIEPAMHR (HA) + eluate addback @ 3x | 0* | 0* | 0* | 0* |
| DIEPAMHR (HA) + 812 mg/L EDTA | 100 | 90 | 88 | 57 |
| DIEPAMHR (HA) + 406 mg/L EDTA | 100 | 97 | 97 | 93 |
| DIEPAMHR (HA) + 1624 mg/L STS | 100 | 86 | 77 | 72 |
| DIEPAMHR (HA) + 812 mg/L STS | 100 | 93 | 93 | 89 |
| DIEPAMHR (HA) + 100 ppb PBO | 83 | 10* | 7* | 7* |
| DIEPAMHR (HA) + 50 ppb PBO | 100 | 32* | 10* | 0* |
| DIEPAMHR (HA) + 500X esterase | 100 | 100 | 100 | 97 |
| DIEPAMHR (HA) air stripped | 100 | 91 | 91 | 91 |
| DIEPAMHR C8 Blank | 100 | 97 | 97 | 76 |
| 723NRBDRY | 30* | 0* | 0* | 0* |
| 723NRBDRY + 812 mg/L EDTA | 20* | 0* | 0* | 0* |
| 723NRBDRY + 406 mg/L EDTA | 27* | 0* | 0* | 0* |
| 723NRBDRY + 203 mg/L EDTA | 17* | 0* | 0* | 0* |
| 723NRBDRY + 1624 mg/L STS | 0* | 0* | 0* | 0* |
| 723NRBDRY + 812 mg/L STS | 0* | 0* | 0* | 0* |
| 723NRBDRY + 406 mg/L STS | 0* | 0* | 0* | 0* |
| 723NRBDRY + 100 ppb PBO | 10* | 0* | 0* | 0* |
| 723NRBDRY + 50 ppb PBO | 0* | 0* | 0* | 0* |
| 723NRBDRY + 500X esterase | 80 | 3* | 0* | 0* |
| 723NRBDRY air stripped | 13* | 0* | 0* | 0* |
| 723NRBDRY C8 Rinsate | 60 | 0* | 0* | 0* |

* indicates survival of less than 50% of the animals in a treatment.

2. This test was set up on 11/09/05.

3. Survival in the laboratory control was 3% below the 90% survival required by USEPA test acceptability criteria.

Table 39. Summary of 48-hr *H. azteca* phase II TIE conducted on sample from 723NRBDRY collected on 10/25/05.[2,3]

| Treatment | 24 hr Survival (%)[1] | | 48 hr Survival (%)[1] | |
|---|---|---|---|---|
| | x | se | x | se |
| DIEPAMHR[P] | 100 | 0.0 | 100 | 0.0 |
| DIEPAMHR + 0.5% MeOH | 100 | 0.0 | 100 | 0.0 |
| DIEPAMHR (HA) + 0.5% of 723NRBDRY 50% Fraction (@ 3x) | 100 | 0.0 | 100 | 0.0 |
| DIEPAMHR (HA) + 0.5% of 723NRBDRY 70% Fraction (@ 3x) | 90 | 5.8 | 73 | 8.8 |
| DIEPAMHR (HA) + 0.5% of 723NRBDRY 75% Fraction (@ 3x) | 20* | 10.0 | 0* | 0.0 |
| DIEPAMHR (HA) + 0.5% of 723NRBDRY 80% Fraction (@ 3x) | 0* | 0.0 | 0* | 0.0 |
| DIEPAMHR (HA) + 0.5% of 723NRBDRY 85% Fraction (@ 3x) | 30* | 5.8 | 0* | 0.0 |
| DIEPAMHR (HA) + 0.5% of 723NRBDRY 90% Fraction (@ 3x) | 96.7 | 3.3 | 43* | 3.3 |
| DIEPAMHR (HA) + 0.5% of 723NRBDRY 95% Fraction (@ 3x) | 100 | 0.0 | 100 | 0.0 |
| DIEPAMHR (HA) + 0.5% of 723NRBDRY 100% Fraction (@ 3x) | 100 | 0.0 | 72 | 3.3 |

P. The laboratory control met the criteria for test acceptability.
* indicates survival of less than 50% of the animals in a treatment.
2. This test was set up on 11/21/05
3. This test was abbreviated to 48 hrs due to the small amount of eluate available.

***Water Chemistry***

Detailed water quality data is presented in Appendix A. Prior to *C. dubia, P. promelas,* and *H. azteca* test initiation and at test termination, temperature, dissolved oxygen (DO), pH, electrical conductivity (EC), alkalinity, hardness, and ammonia were measured. 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.

## Chemical Analyses and TIE Results

Results of chemical analyses performed on selected samples are shown in Table 40. Sample 412LARRHO collected on July 11, 2005 contained 16 TUs of contaminants for *C. dubia* and 8 TUs for *P. promelas*, and TIE results indicated that cationic metals were causing a significant part of the toxicity. The chemical analysis showed that high concentrations of aluminum, copper, zinc, nickel and selenium were present.

Chemical analysis of a water sample collected from site 412LAR024 on July 11, 2005 revealed the presence of two organophosphate insecticides, diazinon (0.05 ppb) and dioxathion (0.4 ppb).

Although the TIE performed on sample 723NRBDRY (sampled May 9, 2005) indicated potential pyrethroid toxicity, the pyrethroid scan did not detect these compounds. According to information received from the analytical lab, it is possible that the sensitivity of the analysis was reduced due to high amounts of oil/grease and other organic compounds in the water sample. A second toxic sample collected from the same site on October 25, 2005, where TIEs indicated that pyrethroids were causing toxicity, was C8 extracted and the column sent for chemical analysis. Two pyrethroids were detected at potentially toxic concentrations: cyfluthrin and permethrin.

Table 40. Results of chemical analyses of samples tested by TIE.

| Region | Sample ID | Sampling Date | Analysis Requested | Concentration (ppb) |
|---|---|---|---|---|
| Region 4 | 412LARRHO | 07/11/05 | dissolved metals scan | Al: 16.60 Ni: 1.00<br>Cr: 0.24 Pb: 0.06<br>Cu: 7.10 Se: 5.32<br>Mn: 0.40 Zn: 5.32 |
| | | | total metals scan | Al: 28.90 Ni: 0.91<br>Cr: 0.29 Pb: 0.13<br>Cu: 7.57 Se: 5.14<br>Mn: 0.81 Zn: 3.47 |
| Region 4 | 412LAR024 | 07/11/05 | pesticide scan | Diazinon: 0.050<br>Dioxathion: 0.400 |
| Region 7 | 723NRBDRY | 05/09/05 | pyrethroid scan | ND |
| Region 7 | 723NRBDRY | 10/25/05 | pesticide scan | Cyfluthrin: 0.013<br>Permethrin: 0.043 |

# SUMMARY AND DISCUSSION

## Region 4

Among 65 water samples from sites in RWQCB Region 4 between April 25 and July 12, 2005, 21 samples (32%) collected from 18 sites (42% of sites) were toxic to *C. dubia* (Table 41). Seven of these samples caused a significant increase in *C. dubia* mortality in addition to a decrease in fecundity (number of neonates). Only one water sample (412 LARRHO; July 11, 2005) caused reduced survival and biomass in fathead minnow larvae, and TIE results revealed that cationic metals, in particular copper, aluminum, selenium, nickel and zinc, were the dominant toxicants. Twelve of the sites where toxicity was detected were in the Los Angeles River watershed (LAR), while only six toxic sites were located in the San Gabriel River watershed (SGB). Since the number of sampling sites was similar between the two watersheds (15 LAR, 15 SGR, 5 upper SGR), these results indicate that toxicity may be more prevalent in the Los Angeles River watershed. The majority of toxic samples were collected in June, but toxicity was also detected throughout the sampling period of April-July 2005.

Several of the toxic samples had a pH >9, and were adjusted to pH <9 in order to verify if high pH levels were responsible for the observed toxicity. For these samples, pH adjustment did not eliminate toxicity, and it must be concluded that stressors other than high pH were causing *C. dubia* toxicity.

Table 41. Toxic samples collected in Region 4 between April 25, 2005 and July 12, 2005

| **Region 4 Site ID** | ***C. dubia* Reproduction** | ***C. dubia* Survival** | ***P. promelas* Growth** | ***P. promelas* Survivial** | **TIE Result** |
|---|---|---|---|---|---|
| | | | | | |
| 412LAR008 | 26-Apr | | | | |
| 412LAR009 | 16-May | | | | |
| 412LAR013 | 21-Jun | | | | |
| 412LAR020 | 16-May | | | | |
| 412LAR024 | 26-Apr | | | | |
| “ * | 11-Jul | 11-Jul | | | Organic compound(s), pyrethroids |
| " pH adj. | 11-Jul | 11-Jul | | | |
| 412LAR025 | 27-Apr | 27-Apr | | | |
| | 16-May | | | | |
| 412LAR031 | 28-Jun | | | | |
| 412LAR031-pH adj. | 28-Jun | | | | |
| 412LARVGO | 13-Jun | 13-Jun | | | |
| 412 LARBLL | 20-Jun | 20-Jun | | | |
| 412 LARBLL-pH adj. | 20-Jun | 20-Jun | | | |
| 412 LARBLL | | | | | |
| 412 LARBLL-pH adj. | 11-Jul | | | | |
| 412LARTJA | 21-Jun | | | | |
| 412LARTJA-pH adj. | 21-Jun | | | | |
| 412LARRHO | 29-Jun | 29-Jun | | | |
| 412LARRHO-pH adj. | 29-Jun | 29-Jun | | | |
| 412LARRHO* | 11-Jul | 11-Jul | 11-Jul | 11-Jul | cationic metal(s), nonpolar organic compound(s), volatile compounds |
| | | | | | |
| 405SGB006 | 16-Jun | 16-Jun | | | |
| 405SGB015 | 24-May | | | | |
| 405SGB022 | 17-May | | | | |
| 405SGBNFK | 15-Jun | | | | |
| “ | 12-Jul | | | | |
| SGUR-01 | 30-Jun | | | | |
| SGUR-02 | 30-Jun | | | | |

* subject to TIE

Water collected on July 11, 2005 from site 412LAR024 was subjected to TIE testing. It contained toxic concentrations (2 TUs) of nonpolar organic compounds, and results indicated that pyrethroid pesticides potentially contributed to the toxicity. Toxicity was removed after C8 extraction, and reduced after the addition of carboxylesterase to the original water sample. Carboxylesterase is an enzyme that rapidly breaks down pyrethroids (Wheelock *et al*. 2005). However, chemical analysis of the water sample could not confirm the presence of pyrethroids (Table 40). Chemical analysis revealed the presence of two organophosphate insecticides, diazinon (0.05 ppb) and dioxathion (0.4 ppb). The LC50 for diazinon is 0.4 ppb, but no information on *C. dubia* toxicity exists for dioxathion. The 96-h LC50 for Atlantic silverside (*Menidia menidia*; fork length: 50 mm) is 6 ppb. For the amphipod *Gammarus fasciatus*, the LC50 is 15 ppb (PAN Pesticide Database, 2006). In general, the toxicity of mixtures of OP pesticides is additive (Bailey et al. 1997).

A sample collected on July 11, 2005 from site 412LARRHO and subject to TIE testing contained toxic amounts (>16 *C. dubia* TUs, 8 fathead minnow TUs) of cationic metal(s), nonpolar organic compound(s) and volatile compounds (e.g. surfactants, ammonia, chlorine). Results of the metals analysis are shown in Table 40.

**Region 7**

Of 15 sites sampled in Region 7 during 2004/2005, eight sites (53%) were toxic at least once during the sampling period (Table 42). Site 713CRNVBD (Colorado River at Nevada State Line) was toxic to *C. dubia* in October 2004 (acute and chronic toxicity) and in October 2005 (chronic toxicity). A sample taken in October 2005 from the Colorado River at Imperial Dam Gates (715CRIDG1) was also chronically toxic to *C. dubia*. Both sampling sites on the New River showed acute *H. azteca* toxicity in May 2005 and again in October 2005. A sample taken from the Coachella Valley Stormchannel (719CVS52, October 2005) was highly toxic to fathead minnow larvae, and caused reduced fecundity in *C. dubia*. The cause of toxicity was determined to be ammonia. Only one other site, 715CPVOD2 (Palo Verde Outfall Drain, October 2005) showed acute fish toxicity as well as reduced fecundity in *C. dubia*. The cause of toxicity in this sample was not determined. A sample taken in October 2005 from Palo Verge Lagoon (715CPVLG1) was also chronically toxic to *C. dubia*. A sample from the

Alamo River Outlet (723ARGRB1, October 2005) was highly toxic (acute and chronic) to *C. dubia*, but the toxicant was not determined.

Table 42. Toxic samples collected in Region 7 between October 2004 and November 2005.

| Region 7 Site ID | *C. dubia* Reproduction | *C. dubia* Survival | *P. promelas* Biomass | *P. promelas* Survivial | *H. azteca* Survival | TIE Result |
|---|---|---|---|---|---|---|
| | | | | | | |
| 713CRNVBD | 10/4/2004 | 10/4/2004 | | | | |
| | 10/24/2005 | | | | | |
| 715CPVLG1 | 10/24/2005 | | | | | |
| 715CPVOD2 | 10/26/2005 | | | 10/26/2005 | | |
| 715CRIDG1 | 10/24/2005 | | | | | |
| 723ARGRB1 | 10/5/2004 | 10/5/2004 | | | | |
| 723NRBDRY* | | | | | 5/10/2005 | pyrethroids, volatile toxic compounds |
| "* | | | | | 10/27/2005 | pyrethroids, OPs |
| 723NROTWM | | | | | 5/10/2005 | |
| " | | | | | 10/27/2005 | |
| 719CVSC52* | 10/27/2005 | | 10/27/2005 | 10/27/2005 | | ammonia |

* subject to TIE

Two TIEs were performed on samples from site 723NRBDRY (New River at Boundary). Results indicated potential pyrethroid toxicity in both samples. In addition, volatile toxic compounds were present in the sample collected in May 2005. Test results on the sample collected in October indicated possible pesticide toxicity. Chemical analysis did not detect pyrethroids in the May 2005 sample, but interference of oil/grease and other organic compounds present in the water sample may have prevented their detection at concentrations toxic to the test organisms. Chemical analysis of the C8 eluate of the October 25, 2005 sample from this site revealed the presence of two pyrethroids at concentrations toxic to invertebrates: Cyfluthrin (0.013 ppb) and Permethrin (0.043 ppb). The LC50s of cyfluthrin for rainbow trout and the opossum shrimp (*Americamysis bahia*) are 0.52 ppb and 0.004 ppb, respectively. For permethrin, the 96-h EC50 for the cladoceran *Daphnia magna* is 0.04 ppb, while the 96-h LC50 for fathead minnow is 23.4 ppb (PAN Pesticide Database, 2006). Overall, the TIE results indicate the presence of pesticides and associated compounds (e.g. surfactants) at toxic concentrations in the New River. Of particular interest is the detection of

pyrethroid pesticides in the water column, because these compounds are believed to sequester to sediments within hours of being transported into surface waters.

## Region 9

Table 43. Toxic samples collected in Region 9 September 2004 - September 2005.

| Region 9 Site ID | *C. dubia* reproduction | *C. dubia* survival | *H. azteca* survival | Algae growth |
|---|---|---|---|---|
| 907SDR15 | 4/19/2005 | | | 9/13/2004 |
| 907SDFRC2 | | | | 9/13/2004 |
| " | | | | 4/19/2005 |
| 907SDALV3 | 2/28/2005 | | | 9/13/2004 |
| 907SDLC02 | 9/13/2004 | | | 9/13/2004 |
| " | 4/19/2005 | | | |
| 907SDSVC4 | 2/28/2005 | | | 9/13/2004 |
| " | 4/19/2005 | | | |
| 907SDCHC3 | 4/19/2005 | | | |
| 903SLGRD2 | 9/14/2004 | | | 9/14/2004 |
| " | 4/18/2005 | | | |
| " | 4/20/2005 | | | 4/20/2005 |
| 903SLSLR8 | 9/14/2004 | | | 9/14/2004 |
| " | 3/1/2005 | | | |
| 903SLMSA2 | | | | 9/14/2004 |
| 903SLKYS3 | 9/14/2004 | | | 9/14/2004 |
| " | 4/20/2005 | | | |
| 900FDQ010 | 9/14/2004 | | | 9/14/2004 |
| " | 6/1/2005 | | | |
| 903SLSLR2 | 9/13/2004 | | | 9/13/2004 |
| 903SLIRS2 | 9/13/2004 | | | |
| 991TTJR05 | 5/31/2005 | | | |
| 911TLAP04 | 6/1/2005 | | | |
| 911TCWD10 | 6/2/2005 | | | |
| 911 TTEC02 | 6/2/2005* | 6/2/2005* | | 6/2/2005 |
| " | | | 9/7/2005 | 9/7/2005 |
| 908SLAW02 | 6/1/2005 | | | |
| 909SSWR08 | | | | 5/31/2005 |
| " | | | | 9/6/2005 |

*toxicity due to ammonia/other toxicants

Among the 22 sampling sites in Region 9, nineteen (86%) exhibited either invertebrate or algal toxicity, or both, in the period September 2004 - September 2005 (Table 43).

At about half of them (nine sites) toxicity was measured more than once during the sampling period: 907SDFRC2 (September 2004, April 2005, algal toxicity only), 907SDLC02 (September 2004, April 2005), 907SDSVC4 (February 2005, April 2005), 903SLGRD2 (September 2004, April 18 and 20, 2005), 903SLSLR8 (September 2004, March 2005), 903 SLKYS3 (September 2004, April 2005), 900FDQ010 (September 2004, June 2005), 911TTEC02 (June 2005, September 2005), and 909SSWR08 (May 2005, September 2005). Only three sites (approx. 16%) were exclusively toxic to algae: 907SDFRC2, 903SLMSA2 and 909SSWR08, and the majority of samples taken in April, May and June 2005 were only toxic to *C. dubia*. Acute (significantly reduced survival) invertebrate toxicity was seen in two samples from site 911TTEC02, one of which was toxic due to ammonia and, in part, other undetermined toxicants.

## QA/QC

UCD ATL tests approximately 10% of all samples for QA/QC determinations. Over the course of this project, 17 samples were selected for QA (approx. 13% - see Table 44). These samples were comprised of the following: 7 field duplicates, 8 bottle blanks, and 2 trip 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 cleaning methods. A trip 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 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 met that requirement with

seven field duplicates. No other applicable QC sample is required by SWAMP for toxicity testing; bottle and trip blanks were included in this project at the discretion of the QA Officer. The overall performance of the QA/QC samples is outlined in Table 44. A more detailed description of QA/QC sample performance can be found in Appendix B.

*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 – the results are considered equivalent. Precision can be measured by calculating the Relative Percent Difference (RPD) between sample measurements. 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 using 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 Appendix B. The frequency of field duplicates sharing equivalent results is outlined in Table 44.

Table 44. Frequency of QA/QC samples sharing equivalent results – entire project

| **Species Endpoint** | **Field Duplicates** | | **Bottle Blanks** | | **Trip Blanks** | |
|---|---|---|---|---|---|---|
| | Sample Size | % Agreement | Sample Size | % Agreement | Sample Size | % Agreement |
| *C. dubia* **survival** | 5 | 100 | 4 | 100 | 1 | 100 |
| *C. dubia* **reproduction** | 5 | 95 | 4 | 100 | 1 | 100 |
| *P. promelas* **survival** | 2 | 100 | 3 | 100 | NA* | NA |
| *P. promelas* **biomass** | 2 | 100 | 3 | 100 | NA | NA |
| *H. azteca* **survival** | 2 | 100 | 0 | 100 | NA** | NA |
| *Algae* **growth** | 3 | 100 | 1 | 100 | 1 | 100 |

* There is no trip blank data for *P. promelas* because the trip blanks were conducted with Region 9, which only utilizes *C. dubia* and *S. capricornutum* species.
** There is no trip blank data for *H. azteca* because that organism is only used with samples which have ECs greater than 2500 umhos, and are used a surrogate for the *C. dubia* species.

*Deviations:* Twelve deviations from the QA/QC plan occurred over the course of the project (see Table 45). These deviations were comprised of the following: exceeded sample temperatures (42%, or 5/12), exceeded holding times (42%, or 5/12), exceeded DO criterion (8%, or 1/12), and deviation from test method (8%, or 1/12).

Warm sample receiving temperatures (> 6º C) were one of the most frequent deviations. The sampling agency was contacted with each occurrence and made aware of the problem. Because this type of deviation occurred frequently during the months of June and July, which are the hottest months in the summer, the warm sample temperatures were most likely due to the ice melting in the coolers during transit. This can be avoided in the future by using larger coolers which can hold more ice to transport the samples, re-icing the coolers before shipping if the samples have sat for more than 24 hours, and/or shipping samples the day of collection.

Exceeding sample holding time was the second most frequent deviation. SWAMP requires that tests are to be initiated within 48 hours of sample collection. Over the course of the project, samples were collected over several days and then shipped together. Often, samples arrived at the laboratory with only a few hours remaining before the 48-hour holding time was reached. This makes meeting holding time requirements very difficult. We recommend shipping each batch of samples on the day of collection instead of shipping multiple sample dates together, as this will increase the likelihood that sample holding times will be met. Moreover, shipping the samples on the day of collection will also help negate the melting of ice in the coolers and aid in meeting sample temperature requirements.

With regard to the exceeded DO criterion, which occurred during the July – August, 2005 quarter, the high sample DO was not expected to impair organism performance. The organisms were exposed to the high DO level for a 24-hour period, and statistical analyses indicated no statistically significant difference between the sample and the laboratory control.

A deviation from method protocols occurred during the September – November, 2005 quarter, with the *H. azteca* Phase II TIE. US EPA (1993) does not specify test duration for a

TIE; however US EPA requires a minimum of two water renewals within a 96-hour period. The *H. azteca* Phase II TIE was conducted as a 48-hour non-renewal test, due to a lack of column eluate. There was a strong organism response within the 48-hour test duration, therefore the resulting data is considered reliable.

Table 45. Deviations from the QA/QC plan

| Region | Number of Deviations | Deviation Type and Frequency | | Overall Occurrence (per Region) |
|---|---|---|---|---|
| 4 | 8 | Holding Time Exceeded: | 3 | 6% |
| | | Sample Temperature Exceeded: | 4 | 8% |
| | | DO Criterion Exceeded: | 1 | 2% |
| 7 | 3 | Holding Time Exceeded: | 2 | 18% |
| | | Deviation from Method: | 1 | 6% |
| 9 | 1 | Sample Temperature Exceeded: | 1 | 2% |

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# APPENDIX A

# APPENDIX A

# QUALITY ASSURANCE/QUALITY CONTROL

## September 30 – December 31, 2004

***Deviations***

In seven September samples (907SSDRI5, 907SDFRC2, 907SDALV3, 907SDLCO2, 903SLSLR8, 903SLMSA2, and 900FDQO10) water quality measurements exceeded the dissolved oxygen (DO) criteria on day three of the *C. dubia* toxicity test initiated on September 15, 2004. Sample measurements ranged from 8.7 to 8.8 mg/L. However, although the physical measurements were greater than 8.6 mg/L, AQUA-Science protocols dictate that a sample's DO parameters are within range if the sample measurement is below 102% saturation. At a temperature of 25° C, the aforementioned DO measurements are below 102% saturation, and are therefore considered to be within the specified range and therefore, acceptable.

***Reference Toxicant Tests***

One reference toxicant test was performed concurrently with each set of toxicity tests conducted to ascertain whether the organism response fell within the acceptable range as dictated by US EPA. For sample dates September 13 -14 2004, *C. dubia* and *S. capricornutum* performed normally in each reference toxicant test. For sample date October 5, 2004, *C. dubia* performed normally in the reference toxicant test. These data indicate that the organisms' response fell within the acceptable range of plus or minus two standard deviations around a running mean, and were responding typically within that range.

***Field Duplicates***

For the months of September and October 2004, field duplicate samples were collected at a rate of 5% to assess precision. These QA/QC samples were collected once per field trip, on September 13 and 14, 2004 for Region 9 (903SLGRD2 and 900FDQO10), and on October 5, 2004 for Region 7 (715CPVLG1 and 700FDQO10). Precision is the degree to which the primary sample agrees with the duplicate sample. Field duplicate samples are in agreement when they both are either statistically similar or statistically different from the control. The frequency of field duplicates sharing equivalent results is outlined in Table A1.

**Table A1. Frequency of field duplicates sharing equivalent results (September 1-December 31, 2004)**

| Quality Assurance Samples | *C. dubia* Mortality | | *C. dubia* Reproduction | | *S. capricornutum* Cell Growth | |
|---|---|---|---|---|---|---|
| | Sample Size | % Agreement | Sample Size | % Agreement | Sample Size | % Agreement |
| Field Duplicates | 2 | 100 | 2 | 100 | 1 | 100 |

Precision for water quality data is assessed by calculating the Relative Percent Difference (RPD) using the following formula:

(100 x { |Duplicate 1 – Duplicate 2| / [Duplicate 1 + Duplicate 2) / 2] } ).

The RPD has been calculated for several water quality parameters and is listed in Tables A2, A3 and A4.

**Table A2. Average Relative Percent Difference (RPD) for field duplicates collected on September 14, 2004 for *C. dubia*.**

| September 14, 2004 (903SLGRD2 and 900FDQO10) *C. dubia* | | | |
|---|---|---|---|
| Parameter | Sample Size (n) | Average % Difference | Standard Deviation |
| DO | 6 | 0.59 | 0.65 |
| pH | 6 | 1.19 | 0.70 |
| Alkalinity | 2 | 5.30 | 4.61 |
| Hardness | 2 | 11.05 | 3.75 |
| EC | 6 | 0.38 | 0.27 |
| Ammonia | 2 | 59.92[A] | 84.74 |

[A] Caution should be applied when interpreting water quality precision data. Although the difference between ammonia replicates is large, it is because low concentrations of ammonia were measured rather than lack of precision.

**Table A3. Average Relative Percent Difference (RPD) for field duplicates collected on September 13 and 14, 2004 for *S. capricornutum*.**

| September 14, 2004 (903SLGRD2 and 900FDQO10) *S. capricornutum* | | | |
|---|---|---|---|
| Parameter | Sample Size (n) | Average % Difference | Standard Deviation |
| DO | 5 | 0.71 | 1.71 |
| pH | 5 | 1.26 | 0.38 |
| EC | 2 | 0.69 | 0.32 |

**Table A4. Average Relative Percent Difference (RPD) for field duplicates collected on October 5, 2004 for *C. dubia*.**

| October 5, 2004 (715CPVLG1 and 700FDQO10) *C. dubia* | | | |
|---|---|---|---|
| Parameter | Sample Size (n) | Average % Difference | Standard Deviation |
| DO | 7 | 0.27 | 0.24 |
| pH | 7 | 0.64 | 0.27 |
| Alkalinity | 2 | 11.11 | 0.95 |
| Hardness | 2 | 5.56 | 2.04 |
| EC | 7 | 0.23 | 0.23 |
| Ammonia | 2 | 44.40[A] | 7.92 |

[A] Caution should be applied when interpreting water quality precision data. Although the difference between ammonia replicates is large, it is because low concentrations of ammonia were measured rather than lack of precision.

***Quality Assurance Audit***

Bill Ray, the Quality Assurance Officer from the SWRCB, visited the UCD ATL on August 5, 2004 for the quality assurance audit. Some of the subjects that were discussed were inter-laboratory split samples, the importance of documenting protocols, elements of the SWAMP QAM, UCD ATL staff proficiency requirements, making QA contacts, and statistics training. Additionally, Bill Ray suggested the development of a 'contingency plan' SOP and the implementation of a program that provides guidance for trainers on the correct method of training.

***Internal Quality Assurance Audit***

Marie Vasi, the UCD ATL Quality Assurance Officer, performed an internal quality assurance audit on the following components: reference toxicant control charts for *C. dubia*, *P. promelas* and *S. capricornutum* from October 2004 and the previous 19 months, staff performance audits, laboratory safety and system audit, UC Davis CUPA Self-Audit, and a chemical inventory audit. Her findings indicate that UCD ATL is current and in compliance in those aspects of the laboratory. Additionally, the Quality Assurance Officer consulted the US EPA audit checklist and found that UCD ATL is in compliance with regards to US EPA criteria. Her audits of these systems did not yield any evidence of deviations or discrepancies.

***Quarterly Performance Audits***

QAPP requirements dictate that any UCD ATL personnel who conduct SWAMP toxicity tests must pass quarterly performance audits with a score of 80% or above. UCD ATL quarterly performance audits consist of a questionnaire which outlines various principles of water quality and toxicity testing. The UCD ATL Quality Assurance Officer arranges individual meetings with each technician to evaluate his or her understanding of those principles. Based on the technician's response to the questionnaire, the UCD ATL Quality Assurance Officer determines whether or not that technician is qualified to

perform SWAMP toxicity testing. All UCD ATL personnel who have performed SWAMP toxicity testing have successfully passed the quarterly performance audits for Water Quality, Internal/QA and Toxicity Testing. Scores for these audits ranged from 83-97%. One employee scored below 80% on the Water Quality audit, at 78.5%. As a new employee, he did not meet the SWAMP training proficiency requirements and therefore did not conduct any SWAMP work at that time. Measures to enhance and/or increase a technician's understanding of the aforementioned principles of water quality and toxicity testing are applied depending on the score of the audit questionnaire. These measures include, but are not limited to, review of UCD ATL protocols and/or SOPs, individual and/or group training refresher courses, and laboratory meetings.

***Staff Proficiency***

All AQUA-Science personnel who performed the September and October toxicity tests have met the proficiency requirements set forth by SWAMP. AQUA-Science personnel who conducted the September and October toxicity tests have three or more years experience conducting US EPA standard three-species toxicity tests, and are therefore considered qualified. All contractual hourly SWAMP proficiency requirements have been met by UCD ATL staff. The new Quality Assurance Officer has 1,356 hours performing the following tasks: investigating control water hardness and alkalinity; conducting Performance and System Audits; developing and revising QAPPs; checking data quality; learning SWAMP QMP and affiliated requirements; attending quality assurance training meetings; report writing (results of quality assurance procedures); training other employees; composing, reviewing and updating Standard Operating Procedures (SOPs); and maintaining reference toxicant control charts.

## January 1 - March 31, 2005

***Deviations***
No deviations occurred during this quarter.

***Reference Toxicant Tests***
One reference toxicant test was performed concurrently with each set of toxicity tests conducted to ascertain whether the organism response fell within the acceptable range as dictated by US EPA. For sample dates February 28 and March 1-2, 2005, *C. dubia* and *S. capricornutum* performed normally within each reference toxicant test. These data suggest that the organisms' response fell within the acceptable range of plus or minus two standard deviations around a running mean, and were responding typically within that range.

***Field Duplicates***
Field duplicate samples were collected at a rate of 5% to assess precision. For this sampling event, these QA/QC samples were collected once, on March 1, 2005 for Regional Board 9 (stations 903SLKYS3 and 900FDQO12, respectively). The frequency of field duplicates sharing equivalent results is outlined in Table A5.

***Bottle Blanks***
Bottle blank samples were included in this sampling event to assess UCD ATL cleaning techniques. Bottle blanks are analyte-free water samples that are transferred to a clean sample container that is prepared in the laboratory. For this project, bottle blanks were comprised of control water for each respective species: SSEPAMH for the *C. dubia* tests, and glass distilled water for the *S. capricornutum* tests. These samples are used to assess potential incidental contamination due to the sample container or cleaning methods. Bottle blank samples are in agreement when they are both either statistically similar or statistically different from the control. The frequency of bottle blanks sharing equivalent results is outlined in Table A5.

***Relative Percent Difference***
Precision for water quality data is assessed by calculating the relative percent difference (RPD) using the following formula:

$$(100 \times \{ |\text{Duplicate 1} - \text{Duplicate 2}| / [\text{Duplicate 1} + \text{Duplicate 2}) / 2] \} ).$$

The RPD has been calculated for several water quality parameters and is listed in Tables A6 and A7.

**Table A5. Frequency of QA samples sharing equivalent results**

| Quality Assurance Samples | *C. dubia* Mortality | | *C. dubia* Reproduction | | *S. capricornutum* Cell Growth | |
|---|---|---|---|---|---|---|
| | Sample Size | % Agreement | Sample Size | % Agreement | Sample Size | % Agreement |
| Field Duplicates | 2 | 100 | 2 | 100 | 2 | 100 |
| Bottle Blanks | 1 | 100 | 1 | 100 | 1 | 100 |

**Table A6. Average Relative Percent Difference (RPD) for field duplicates collected on March 1, 2005 for *C. dubia*.**

| March 1, 2005 (stations 903SLKYS3 and 900FDQO12) *C. dubia* | | | |
|---|---|---|---|
| Parameter | Sample Size (n) | Average % Difference | Standard Deviation |
| DO | 8 | 0.81 | 0.97 |
| pH | 8 | 0.08 | 0.09 |
| Alkalinity | 2 | 3.21 | 1.97 |
| Hardness | 2 | 1.50 | 0.69 |
| EC | 2 | 1.40 | 0.59 |
| Ammonia | 2 | 5.00 | 7.07 |

**Table A7. Average Relative Percent Difference (RPD) for field duplicates collected on March 1, 2005 for *S. capricornutum*.**

| March 1, 2005 (stations 903SLKYS3 and 900FDQO12) *S. capricornutum* | | | |
|---|---|---|---|
| Parameter | Sample Size (n) | Average % Difference | Standard Deviation |
| DO | 2 | 1.16 | 0.08 |
| pH | 5 | 0.33 | 0.43 |
| Alkalinity | 2 | 0.91 | 1.29 |
| Hardness | 2 | 1.87 | 0.16 |
| EC | 2 | 1.08 | 0.31 |
| Ammonia | 2 | 33.33[A] | 47.14 |

[A] Caution should be applied when interpreting water quality precision data. Although the difference between ammonia replicates is large, it is because low concentrations of ammonia were measured rather than lack of precision.

***Internal Quality Assurance Audits***

The UCD ATL Quality Assurance Officer performed an internal quality assurance audit on the following components: reference toxicant control charts for *C. dubia* and *S. capricornutum* from March 2005 and the previous 19 months, staff performance audits, laboratory safety and systems audit. Her findings indicate that UCD ATL is current and in compliance in those aspects of the laboratory. Additionally, the Quality Assurance Officer consulted the US EPA audit checklist and found that UCD ATL is in compliance with regards to US EPA criteria. Her audits of these systems did not yield any evidence of deviations or discrepancies.

***Quarterly Staff Performance Audits***

All UCD ATL personnel who had performed SWAMP toxicity testing have successfully passed the quarterly performance audits for Water Quality, Internal/QA, and Toxicity Testing. Scores for these audits ranged from 81-100%. Measures to enhance and/or increase a technician's understanding of the aforementioned principles of water quality and toxicity testing are applied depending on the score of the audit questionnaire. These measures include, but are not limited to, review of UCD ATL protocols and/or SOPs, individual and/or group training refresher courses, and laboratory meetings.

## April 1-June 30, 2005

***Deviations***
Seven deviations from the QA/QC plan for toxicity testing occurred this past quarter and are outlined below.

**Table A8. Deviations from the QA/QC plan for toxicity testing**

| *Region* | *Date* | *Deviation Type* | *Reason for Deviation* |
|---|---|---|---|
| 4 | 051805 | 48-hour holding time exceeded for test initiation | Neonates were not born in time to meet holding time requirements for *C. dubia* test. |
| 4 | 051805 | 48-hour holding time exceeded for test initiation | Dissolved oxygen levels did not drop to acceptable limits to meet holding time requirements for *P. promelas* test. |
| 4 | 052505 | 48-hour holding time exceeded for test initiation | A high-EC sample was not expected. *H. azteca* had to be ordered last minute. |
| 4 | 062205 | Sample 412LAR016 arrived at the lab at 8.1° C. | Ice melted in transit due to hot weather. |
| 4 | 062905 | Sample 412LAR031 arrived at the lab at 11.6° C. | Ice melted in transit due to hot weather. |
| 7 | 051605 | 48-hour holding time exceeded for ammonia | Sample was overlooked; technician error. |
| 9 | 060305 | Sample 911TCWD10 arrived at the lab at 10.4° C. | Ice melted in transit due to hot weather; courier service delivered samples late. |

***Sample Temperatures***
No significant loss of toxicity is expected due to temperatures exceeding 6° C. The highest temperature was 11.6° C so, in our professional opinion, there was little or no significant degradation of toxicants.

***Sample Holding Time***
The tests that exceeded the 48-hour holding time were setup within the EPA maximum holding time of 72-hours. We doubt that any toxicity was lost and believe that the toxicity test results are reliable.

***Ammonia Holding Time***
The ammonia holding time was exceeded by 96 hours for sample 723ARGRB1 collected in Region 7. Over this period of time, some ammonia degradation possibly occurred. However, a field duplicate was collected at site 723ARGRB1. An ammonia reading was taken on this sample within the holding time, and it was well below the test organisms' tolerance level. In our professional judgment the test results for this site are reliable and ammonia levels would not have impacted the test organisms.

***Test Acceptability Criteria***
Two *P. promelas* tests did not meet test acceptability criteria for biomass. Test acceptability criteria require a minimum of 80% survival and an average dry biomass of ≥0.250 mg in the control. For test initiation date May 26, 2005 for Region 4, average biomass for the control was 0.243 mg, and for test initiation date June 10, 2005 for Region 4, average biomass for the control was 0.217 mg. Both controls met test acceptability criteria for survival, at 91.25 and 95.0%, respectively.

Biomass toxicity is rarely seen in *P. promelas* chronic toxicity tests. Generally, if the fish do not experience high mortality levels, they have biomass levels that are similar to the controls. All of the fish in the test (in ambient samples and in the controls) had similar biomasses. Survival was above 80% in all of the samples tested in the two fish tests. Tests conducted within the same week as the two fish tests that failed passed EPA test acceptability criteria for biomass and survival. Due to these results, we do not believe the low biomass was due to control water, feeding, or technician error. Most likely, the fish were handled too roughly during shipment and, although they appeared healthy, experienced lower levels of growth. In our professional opinion the data are reliable due to the high survival rate in the control and the ambient samples.

***Reference Toxicant Tests***
One reference toxicant test was performed concurrently with each initial set of toxicity tests conducted to ascertain whether the organism response fell within the acceptable range as dictated by US EPA. For the months of April, May and June, *C. dubia, P. promelas, H. azteca* and *S. capricornutum* performed normally within each reference toxicant test. These data suggest that the organisms' response fell within the acceptable range of plus or minus two standard deviations around a running mean, and are responding typically within that range.

***Field Duplicates***
Field duplicate samples were collected at a rate of 5% to assess precision. For this quarter, these QA/QC samples were collected on: April 26, 2005 for Region 9 (400FDQ017 and 412LAR008, respectively); May 9, 2005 for Region 7 (700FDQ011 and 723ARGB1, respectively); and June 1, 2005 for Region 9 (900FDQ014 and 911TLAP04, respectively). The frequency of field duplicates sharing equivalent results is outlined in Table A9.

***Bottle Blanks***
Bottle blank samples were included in this quarter to assess UCD ATL cleaning techniques. The frequency of bottle blanks sharing equivalent results is outlined in Table A9.

***Trip Blanks***
Trip blank samples were included in this quarter to assess potential contamination issues in the field. Trip blanks are analyte-free water samples that are transferred to a clean sample container that is prepared in the laboratory. These samples are taken out into the field and treated like any other collected sample throughout the course of the day. For this project, trip blanks were comprised of control water for each respective species: SSEPAMH for the *C. dubia* tests, and Glass Distilled water for the *S. capricornutum* tests. Trip blank samples are in agreement when they do not differ statistically. The frequency of trip blanks sharing equivalent results is outlined in Table A9.

***Relative Percent Difference***
Precision for water quality data is assessed by calculating the relative percent difference between a QA sample and its equivalent. The RPD has been calculated for several water quality parameters and is listed in Tables A10 – A15.

**Table A9. Frequency of QA samples sharing equivalent results**

| Quality Assurance Samples | *C. dubia* Mortality | | *C. dubia* Reproduction | | *S. capricornutum* Cell Growth | |
|---|---|---|---|---|---|---|
| | Sample Size | % Agreement | Sample Size | % Agreement | Sample Size | % Agreement |
| Field Duplicates | 4 | 100 | 4 | 75[1] | 2 | 100 |
| Bottle Blanks | 4 | 100 | 4 | 100 | 0 | NAP |
| Trip Blanks | 2 | 100 | 2 | 100 | 2 | 100 |

| Quality Assurance Samples | *P. promelas* Mortality | | *P. promelas* Biomass | | *H. azteca* Mortality | |
|---|---|---|---|---|---|---|
| | Sample Size | % Agreement | Sample Size | % Agreement | Sample Size | % Agreement |
| Field Duplicates | 4 | 100 | 4 | 100 | 2 | 100 |
| Bottle Blanks | 4 | 100 | 4 | 100 | 0 | NAP |

[1] For sample date April 26, 2005, the site (412LAR008) was found to be significantly different from the control in reproduction, however the field duplicate (400FDQ017) was not found to be significantly different from the control in reproduction. In this instance, the samples are considered to be on the border between toxic and non-toxic, whereas one was detected as toxic, and the other was not. An additional student's t-test was run to compare the site with its duplicate, and the sites were found to be equivalent to each other ($P = 0.6748$).

**Table A10. Average Relative Percent Difference (RPD) for field duplicates collected on April 26, 2005 for *C. dubia***

| April 26, 2005 (400FDQ017 and 412LAR008) *C. dubia* | | | |
|---|---|---|---|
| Parameter | Sample Size (n) | Average % Difference | Standard Deviation |
| DO | 7 | 11.14 | 4.46 |
| pH | 7 | 0.62 | 0.62 |
| Alkalinity | 2 | 1.70 | 0.94 |
| Hardness | 2 | 2.87 | 1.86 |
| EC | 2 | 0.68 | 0.56 |
| Ammonia | 2 | 5.27 | 7.44 |

**Table A11. Average Relative Percent Difference (RPD) for field duplicates collected on April 26, 2005 for *P. promelas***

| April 26, 2005 (400FDQ017 and 412LAR008) *P. promelas* | | | |
|---|---|---|---|
| Parameter | Sample Size (n) | Average % Difference | Standard Deviation |
| DO | 8 | 4.28 | 2.94 |
| pH | 8 | 1.70 | 1.48 |
| Alkalinity | 2 | 2.25 | 0.17 |
| Hardness | 2 | 0.78 | 1.10 |
| EC | 2 | 1.10 | 0.05 |
| Ammonia | 2 | 17.95 | 25.38 |

**Table A12. Average Relative Percent Difference (RPD) for field duplicates collected on May 9, 2005 for *P. promelas***

| May 9, 2005 (700FDQ011 and 723ARGB1) *P. promelas* | | | |
|---|---|---|---|
| Parameter | Sample Size (n) | Average % Difference | Standard Deviation |
| DO | 8 | 1.48 | 2.08 |
| pH | 8 | 0.40 | 0.28 |
| Alkalinity | 2 | 0.93 | 1.31 |
| Hardness | 2 | 4.17 | 0.25 |
| EC | 2 | 0.83 | 0.15 |
| Ammonia | 2 | 18.22 | 25.77 |

**Table A13. Average Relative Percent Difference for field duplicates collected on May 9, 2005 for *H. azteca***

| May 9, 2005 (700FDQ011 and 723ARGB1) *H. azteca* | | | |
|---|---|---|---|
| Parameter | Sample Size (n) | Average % Difference | Standard Deviation |
| DO | 3 | 6.39 | 4.75 |
| pH | 3 | 1.08 | 0.28 |
| Alkalinity | 2 | 2.89 | 1.46 |
| Hardness | 2 | 6.84 | 4.85 |
| EC | 2 | 2.75 | 3.78 |
| Ammonia | 2 | 18.23 | 25.76 |

**Table A14. Average Relative Percent Difference (RPD) for field duplicates collected on June 1, 2005 for *C. dubia***

| June 1, 2005 (900FDQ014 and 911TLAP04) *C. dubia* | | | |
|---|---|---|---|
| Parameter | Sample Size (n) | Average % Difference | Standard Deviation |
| DO | 8 | 0.99 | 0.91 |
| pH | 8 | 0.12 | 0.22 |
| Alkalinity | 2 | 1.00 | 0.38 |
| Hardness | 2 | 10.77 | 13.28 |
| EC | 2 | 1.26 | 0.06 |
| Ammonia | 2 | 11.76 | 16.64 |

**Table A15. Average Relative Percent Difference (RPD) for field duplicates collected on June 1, 2005 for *S. capricornutum***

| June 1, 2005 (900FDQ014 and 911TLAP04) *S. capricornutum* | | | |
|---|---|---|---|
| Parameter | Sample Size (n) | Average % Difference | Standard Deviation |
| DO | 5 | 1.82 | 0.84 |
| pH | 5 | 0.23 | 0.13 |
| Alkalinity | 2 | 3.70 | 3.43 |
| Hardness | 2 | 6.71 | 7.84 |
| EC | 2 | 1.48 | 0.63 |
| Ammonia | 2 | 0.00 | 0.00 |

***Internal Quality Assurance Audits***
The UCD ATL QA Officer performed an internal quality assurance audit on the following components: reference toxicant control charts for *C. dubia, P. promelas, H. azteca* and *S. capricornutum* from June 2005 and the previous 19 months, staff performance audits, laboratory safety and systems audit. Her findings indicate that UCD ATL is current and in compliance in those aspects of the laboratory. Additionally, the QA Officer consulted the US EPA audit checklist and found that UCD ATL is in compliance with regards to US EPA criteria. Her audits of these systems did not yield any evidence of deviations or discrepancies.

***Quarterly Staff Performance Audits***
For this quarter, individual meetings were not feasible due to time constraints with the intense sampling schedule. In lieu of the individual meetings, UCD ATL technicians were given a two-page test with true/false questions pulled from the original questionnaire. All UCD ATL personnel who have performed SWAMP toxicity testing have successfully passed the quarterly performance audits for Water Quality, Internal/QA and Toxicity Testing. Scores for these audits ranged from 81-100%. Measures to enhance and/or increase a technician's understanding of the aforementioned principles of water quality and toxicity testing are applied depending on the score of the audit test. These measures include, but are not limited to, review of UCD ATL protocols and/or SOPs, individual and/or group training refresher courses, and laboratory meetings.

## July 1 – August 31, 2005

***Deviations***
Three deviations from the QA/QC plan for toxicity testing occurred this past quarter and are outlined below.

**Table A16. Deviations from the QA/QC plan for toxicity testing**

| *Region* | *Date* | *Deviation Type* | *Reason for Deviation* |
|---|---|---|---|
| 4 | 070105 | Sample SGUR-65 arrived at the lab at 16.5° C; Sample SGUR-01 arrived at the lab at 19.6° C; Sample SGUR-02 arrived at the lab at 19.4° C. | Ice melted in transit due to hot weather. |
| 4 | 071305 | Sample 405SGBNFK arrived at the lab at 12.5° C. | Ice melted in transit due to hot weather. |
| 4 | 071305 | Dissolved Oxygen criterion was exceeded at 9.1 mg/L. | Sample was overlooked; technician error. |

***Sample Temperatures***
No significant loss of toxicity is expected due to temperatures exceeding 6° C. The highest temperature was 19.6° C, therefore in our professional opinion there was little or no significant degradation of toxicants.

***Dissolved Oxygen***
The dissolved oxygen (DO) criterion was exceeded for sample 412LARBLL upon test initiation. The DO level could have impacted the test organisms. However, this is unlikely because the organisms were exposed to the high DO level for a 24-hour period. In our professional opinion the test results for this site are reliable.

***Test Acceptability Criteria***
One *P. promelas* toxicity identification evaluation (TIE) test did not meet test acceptability criteria for survival. Test acceptability criteria require a minimum of 90% survival in an acute test. For TIE test initiation date July 15, 2005 for Region 4, average survival for the control was 85%.

An additional control was included that was hardness-adjusted to match that of the ambient sample. This treatment met test acceptability criteria for survival at 95%. Moreover, the primary control's average survival was within 5% of the US EPA criterion. In our professional opinion the data are reliable due to the high survival rate in the hardness-adjusted control and the method blanks.

***Reference Toxicant Tests***
One reference toxicant test was performed concurrently with each initial set of toxicity tests conducted to ascertain whether the organism response fell within the acceptable range as dictated by US EPA. For the months of July, August and September, *C. dubia, P. promelas, H. azteca* and *S. capricornutum* performed normally within each reference toxicant test. These data suggest that the organisms' response fell within the acceptable range of plus or minus two standard deviations around a running mean, and are responding typically within that range.

***Field duplicates***
There were no field duplicates sampled for this quarter.

***Bottle blanks***
There were no bottle blanks tested for this quarter.

***Trip blanks***
There were no trip blanks tested for this quarter.

***Internal Quality Assurance Audits***
The UCD ATL QA Officer performed an internal quality assurance audit on the following components: reference toxicant control charts for *C. dubia, P. promelas, H. azteca* and *S. capricornutum* from September 2005 and the previous 19 months, staff performance audits, laboratory safety and systems audit. Her findings indicate that UCD ATL is current and in compliance in those aspects of the laboratory. Her audits of these systems did not yield any evidence of deviations or discrepancies.

***Quarterly Staff Performance Audits***
For this quarter, in lieu of individual meetings, UCD ATL technicians were given a two-page test with true/false questions pulled from the original questionnaire. All UCD ATL personnel who have performed SWAMP toxicity testing have successfully passed the quarterly performance audits for Water Quality, Internal/QA and Toxicity Testing. Scores for these audits ranged from 82-87 %. Measures to enhance and/or increase a technician's understanding of the aforementioned principles of water quality and toxicity testing are applied depending on the score of the audit test. These measures include, but are not limited to, review of UCD ATL protocols and/or SOPs, individual and/or group training refresher courses, and laboratory meetings.

## September 1 – November 30, 2005

***Deviations***

Two deviations from the QA/QC plan for toxicity testing occurred this past quarter and are outlined below.

**Table A17. Deviations from the QA/QC plan for toxicity testing**

| *Region* | *Date* | *Deviation Type* | *Reason for Deviation* |
|---|---|---|---|
| 7 | 110905 | *H. azteca* Phase I TIE was not initiated within 48 hours of detected toxicity. | Laboratory staff was unable to make contact with the appropriate person to determine follow-up procedures; technical difficulties. |
| 7 | 112105 | *H. azteca* Phase II TIE was conducted as a 48-hour non-renewal test. | There was not enough eluate available for daily water renewals. |

***Phase I TIE Sample Holding Time***

The Phase I TIE test that exceeded the 48-hour test initiation time was set up within ten days of detected toxicity. Toxicity was detected on Sunday, October 30th. UCD ATL staff did not get confirmation to proceed with testing until Tuesday, November 1st. The TIE test was scheduled to be initiated on Sunday, November 6th; however laboratory staff was unable to successfully make the hardness-adjusted control water needed to conduct the TIE until Wednesday, November 9th. Given the span of time between the detected toxicity in the initial screening test and the initiation of the TIE, it is likely that some toxicity may have been lost in the original ambient sample. The original sample was retested in the TIE, and exhibited 70% mortality within 24 hours of test initiation, and 100% mortality by 48 hours. While some toxic units (TUs) of the sample may have degraded during the extended holding time, there were enough present to exhibit a strong response in the TIE. In our professional opinion, we believe that the data is reliable.

***Abbreviated Phase II TIE***

According to US EPA (1993), Phase II TIEs are conducted with a minimum of two water renewals within a 96-hour test duration. After conducting the Phase I TIE, and sending an SPE column for chemical analysis, only one SPE column was left to conduct a Phase II TIE when two columns were needed. The Phase II TIE was initiated as a 48-hour non-renewal test, using the eluate that was available. The 80% methanol fraction exhibited 100% mortality within 24 hours, and the 75% and 85% methanol fractions exhibited 100% mortality within 48 hours. Due to the strong organism responses within the 48-hour test, we believe that the data is reliable.

***Test Acceptability Criteria***
One *H. azteca* Phase I TIE did not meet test acceptability criteria for survival. Acute test acceptability criteria require a minimum of 90% survival in the control. For the Phase I TIE, initiated on November 9, 2005, for Region 7, average survival in the control was 87%. An additional control was included that was hardness-adjusted to match that of the ambient sample. This treatment met test acceptability criteria for survival at 97%. Moreover, the primary control's average survival was within 3% of the US EPA criterion. In our professional opinion the data are reliable due to the high survival rate in the hardness-adjusted control.

***Reference Toxicant Tests***
One reference toxicant test was performed concurrently with each initial set of toxicity tests conducted to ascertain whether the organism response fell within the acceptable range as dictated by US EPA. For the months of September, October and November, *C. dubia, P. promelas, H. azteca* and *S. capricornutum* performed normally within each reference toxicant test. These data suggest that the organisms' response fell within the acceptable range of plus or minus two standard deviations around a running mean, and are responding typically within that range.

***Field Duplicates***
For the month of October 2005, field duplicate samples were collected at a rate of 5% to assess precision. These QA/QC samples were collected once, on October 26, 2005 for Region 7 (700FDQ012 and 723NROTWM). The frequency of field duplicates sharing equivalent results is outlined in below.

**Table A18. Frequency of field duplicates sharing equivalent results**

| Quality Assurance Samples | *H. azteca* Mortality | |
|---|---|---|
| | Sample Size | % Agreement |
| Field Duplicates | 2 | 100 |

***Relative Percent Difference***
Precision for water quality data is assessed by calculating the relative percent difference between a QA sample and its equivalent. The RPD has been calculated for several water quality parameters and is listed in Table A19.

**Table A19. Average Relative Percent Difference (RPD) of field duplicate collected on October 26, 2005 for *H. azteca.***

| October 26, 2005 (700FDQ012 and 723NROTWM) *H. azteca* | | | |
|---|---|---|---|
| Parameter | Sample Size (n) | Average % Difference | Standard Deviation |
| DO | 3 | 5.22 | 9.04 |
| pH | 3 | 1.97 | 2.79 |
| Alkalinity | 2 | 15.20 | 20.51 |
| Hardness | 2 | 1.59 | 2.24 |
| EC | 2 | 1.56 | 0.94 |
| Ammonia | 2 | 5.26 | 7.44 |

***Bottle Blanks***
Bottle blank samples were included in this quarter to assess UCD ATL cleaning techniques. The frequency of bottle blanks sharing equivalent results is listed in Table A20.

**Table A20. Frequency of bottle blanks sharing equivalent results**

| Quality Assurance Samples | *C. dubia* Mortality | | *C. dubia* Reproduction | |
|---|---|---|---|---|
| | Sample Size | % Agreement | Sample Size | % Agreement |
| Bottle Blanks | 2 | 100 | 2 | 100 |

| | *P. promelas* Mortality | | *P. promelas* Biomass | |
|---|---|---|---|---|
| Bottle Blanks | Sample Size | % Agreement | Sample Size | % Agreement |
| | 2 | 100 | 2 | 100 |

***Trip blanks***
There were no trip blanks tested for this quarter.

***Internal quality assurance audits***
No additional internal quality assurance audits were conducted this quarter.

***Quarterly staff performance audits***
No additional staff performance audits were conducted this quarter.

***Project Completeness***

Completeness is a measure of the data obtained compared to the amount of data expected in a project. UCD ATL strives for a minimum of 90% completion of data. Of the 86 toxicity tests conducted for this project, 81 tests met all US EPA test acceptability criteria. Five tests did not meet EPA test acceptability criteria for the following reasons: One *S. capricornutum* test did not meet the % CV criterion; two *P. promelas* tests did not meet biomass criterion, One *P. promelas* Phase I TIE did not meet the survival criterion, and one *H. azteca* Phase I TIE did not meet the survival criterion. By these results, UCD ATL has reached a 94% completeness of data for the entire project.

***Project Relative Percent Difference***

Precision for water quality data is assessed by calculating the relative percent difference (RPD) of measurements taken between field duplicates. The average RPD between field duplicates was calculated for water quality data such as DO, pH, EC, hardness, alkalinity, and ammonia, and is listed in the previous Tables A2-A4, A6, A7, A10-A15, and A19. The average RPD is calculated by taking the average of the individual RPD measurements per field duplicate. The individual RPD between field duplicate measurements is listed below, in Tables A21 through A26.

**Table A21. Relative Percent Difference (RPD) for Electrical Conductivities of Field Duplicates**

| Sample Date | **900FDQ010** (Sept. 14, 2004) | **700FDQ010** (Oct. 5, 2004) | **900FDQ012** (March 1, 2005) | **400FDQ017** (Apr. 26, 2005) | **700FDQ011** (May 9, 2005) | **900FDQ014** (June 1, 2005) | **700FDQ012** (Oct. 26, 2005) |
|---|---|---|---|---|---|---|---|
| Species | **Relative Percent Difference** | | | | | | |
| ***C. dubia*** | 0.55 | 0.24 | 1.82 | 1.07 | | 1.30 | |
| | 0.51 | 0.08 | 0.98 | 0.28 | | 1.22 | |
| | 0.05 | 0.43 | | | | | |
| | 0.26 | 0.21 | | | | | |
| | 0.76 | 0.03 | | | | | |
| | 0.15 | 0.64 | | | | | |
| | | 0.01 | | | | | |
| ***P. promelas*** | | | | 1.07 | 0.73 | | |
| | | | | 1.14 | 0.94 | | |
| ***H. azteca*** | | | | | 0.08 | | 2.23 |
| | | | | | 5.42 | | 0.90 |
| ***S. capricornutum*** | 0.92 | | 1.30 | | | 1.93 | |
| | 0.46 | | 0.86 | | | 1.04 | |

**Table A22. Relative Percent Difference (RPD) for pH of Field Duplicates**

| Sample Date | **900FDQ010** (Sept. 14, 2004) | **700FDQ010** (Oct. 5, 2004) | **900FDQ012** (March 1, 2005) | **400FDQ017** (Apr. 26, 2005) | **700FDQ011** (May 9, 2005) | **900FDQ014** (June 1, 2005) | **700FDQ012** (Oct. 26, 2005) |
|---|---|---|---|---|---|---|---|
| Species | **Relative Percent Difference** | | | | | | |
| ***C. dubia*** | 1.55 | 0.59 | 0.24 | 1.94 | | 0.62 | |
| | 0.13 | 0.43 | 0.00 | 0.25 | | 0.00 | |
| | 1.54 | 0.98 | 0.00 | 0.64 | | 0.00 | |
| | 0.50 | 0.26 | 0.12 | 0.12 | | 0.12 | |
| | 1.65 | 0.97 | 0.00 | 0.25 | | 0.24 | |
| | 1.79 | 0.70 | 0.12 | 0.62 | | 0.00 | |
| | | 0.76 | 0.00 | 0.50 | | 0.00 | |
| | | | 0.12 | | | 0.00 | |
| ***P. promelas*** | | | | 2.10 | 0.13 | | |
| | | | | 3.28 | 0.88 | | |
| | | | | 2.76 | 0.12 | | |
| | | | | 3.76 | 0.37 | | |
| | | | | 1.23 | 0.73 | | |
| | | | | 0.25 | 0.25 | | |
| | | | | 0.13 | 0.49 | | |
| | | | | 0.12 | 0.24 | | |
| ***H. azteca*** | | | | | 1.37 | | 0.00 |
| | | | | | 1.03 | | |
| | | | | | 0.83 | | |
| ***S. capricornutum*** | 1.41 | | 0.00 | | | 0.25 | |
| | 1.18 | | 0.00 | | | 0.12 | |
| | 1.28 | | 0.35 | | | 0.12 | |
| | 0.69 | | 1.05 | | | 0.23 | |
| | 1.75 | | 0.23 | | | 0.44 | |

**Table A23. Relative Percent Difference (RPD) for Dissolved Oxygen of Field Duplicates**

| Sample Date | **900FDQ010** (Sept.14, 2004) | **700FDQ010** (Oct.5, 2004) | **900FDQ012** (Mar. 1, 2005) | **400FDQ017** (Apr. 26, 2005) | **700FDQ011** (May 9, 2005) | **900FDQ014** (June 1, 2005) | **700FDQ012** (Oct. 26, 2005) |
|---|---|---|---|---|---|---|---|
| Species | **Relative Percent Difference** | | | | | | |
| ***C. dubia*** | 0.00 | 0.31 | 1.20 | 3.59 | | 2.50 | |
| | 1.21 | 0.00 | 1.34 | 16.77 | | 0.00 | |
| | 0.00 | 0.30 | 2.60 | 8.92 | | 1.31 | |
| | 1.16 | 0.30 | 0.00 | 13.16 | | 1.32 | |
| | 0.00 | 0.30 | 0.00 | 13.33 | | 0.00 | |
| | 1.18 | 0.00 | 1.36 | 14.01 | | 1.44 | |
| | | 0.71 | 0.00 | 8.21 | | 1.38 | |
| | | | 0.00 | | | 0.00 | |
| ***P. promelas*** | | | | 1.17 | 0.00 | | |
| | | | | 3.77 | 0.00 | | |
| | | | | 1.24 | 1.31 | | |
| | | | | 2.67 | 1.36 | | |
| | | | | 7.09 | 6.35 | | |
| | | | | 6.80 | 1.38 | | |
| | | | | 7.19 | 1.42 | | |
| | | | | 0.00 | 0.00 | | |
| ***H. azteca*** | | | | | 1.24 | | 0.00 |
| | | | | | 10.61 | | 0.00 |
| | | | | | 7.33 | | |
| ***S. capricornutum*** | 0.00 | | 1.21 | | | 1.23 | |
| | 2.41 | | 1.10 | | | 2.41 | |
| | 0.00 | | | | | | |
| | 0.00 | | | | | | |
| | 1.14 | | | | | | |

**Table A24. Relative Percent Difference (RPD) for Hardness of Field Duplicates**

| Sample Date | **900FDQ010** (Sept.14, 2004) | **700FDQ010** (Oct.5, 2004) | **900FDQ012** (March 1, 2005) | **400FDQ017** (Apr. 26, 2005) | **700FDQ011** (May 9, 2005) | **900FDQ014** (Jun. 1, 2005) | **700FDQ012** (Oct. 26, 2005) |
|---|---|---|---|---|---|---|---|
| | **Relative Percent Difference** | | | | | | |
| **Hardness** | 8.40 | 4.11 | 1.99 | 1.55 | 3.99 | 1.38 | 3.17 |
| | 13.70 | 7.00 | 1.02 | 4.18 | 4.35 | 20.15 | 0.00 |

**Table A25. Relative Percent Difference (RPD) for Alkalinity of Field Duplicates**

| Sample Date | **900FDQ010** (Sept.14, 2004) | **700FDQ010** (Oct.5, 2004) | **900FDQ012** (March 1, 2005) | **400FDQ017** (Apr. 26, 2005) | **700FDQ011** (May 9, 2005) | **900FDQ014** (June 1, 2005) | **700FDQ012** (Oct. 26, 2005) |
|---|---|---|---|---|---|---|---|
| | **Relative Percent Difference** | | | | | | |
| **Alkalinity** | 2.04 | 11.70 | 1.82 | 2.37 | 1.85 | 1.27 | 0.70 |
| | 8.56 | 10.40 | 4.61 | 1.04 | 0.00 | 0.73 | 29.70 |

**Table A26. Relative Percent Difference (RPD) for Ammonia of Field Duplicates**

| Sample Date | **900FDQ010** (Sept.14, 2004) | **700FDQ010** (Oct.5, 2004) | **900FDQ012** (March 1, 2005) | **400FDQ017** (Apr. 26, 2005) | **700FDQ011** (May 9, 2005) | **900FDQ014** (June 1, 2005) | **700FDQ012** (Oct. 26, 2005) |
|---|---|---|---|---|---|---|---|
| | **Relative Percent Difference** | | | | | | |
| **Ammonia** | 0.00 | 38.79 | 0.00 | 0.01 | 36.44 | 0.00 | 0.00 |
| | 119.00 | 50.00 | 10.00 | 10.53 | 0.01 | 23.50 | 10.53 |

* Please note that caution should be applied when interpreting water quality precision data. Although the difference between ammonia replicates is large, it is because low concentrations of ammonia were measured rather than lack of precision.

***Reference Toxicant Control Charts***

The following charts outline organism performance at UCD ATL from November, 2005 and the previous 19 months. Over the course of the entire project there were no outliers, and the organisms' performance was considered normal.

**FigureA1. Control chart for *C. dubia* survival**

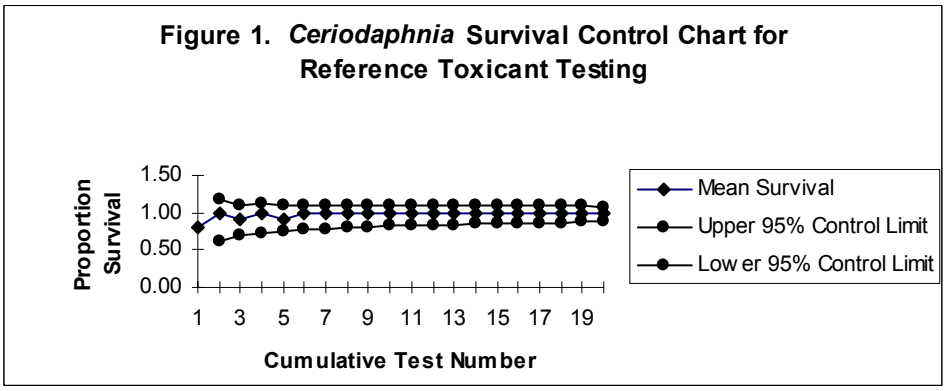



**Figure A2. Control chart for *C. dubia* reproduction**

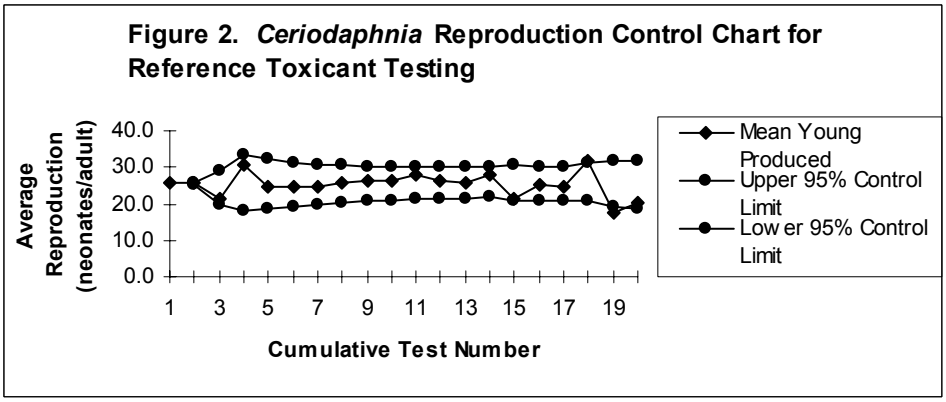



**Figure A3. Control chart for *P. promelas* survival**

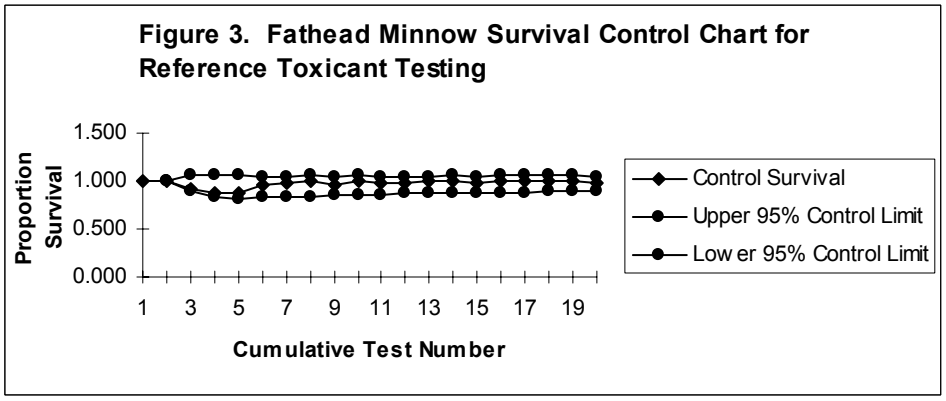


**Figure A4. Control chart for *P. promelas* biomass**

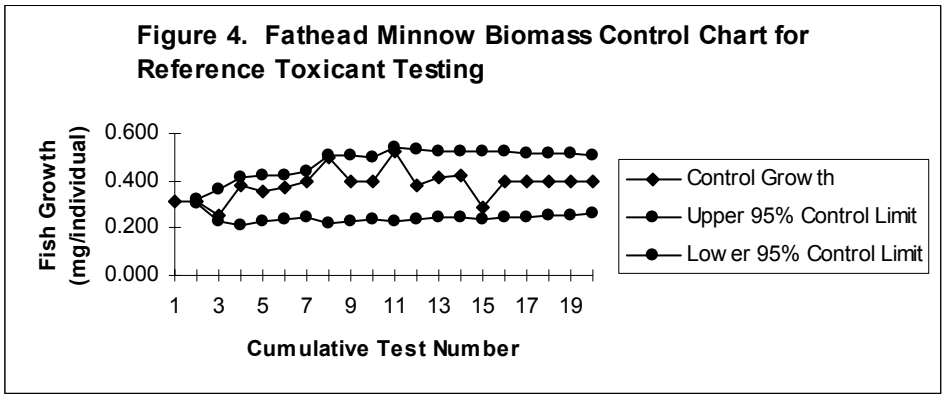
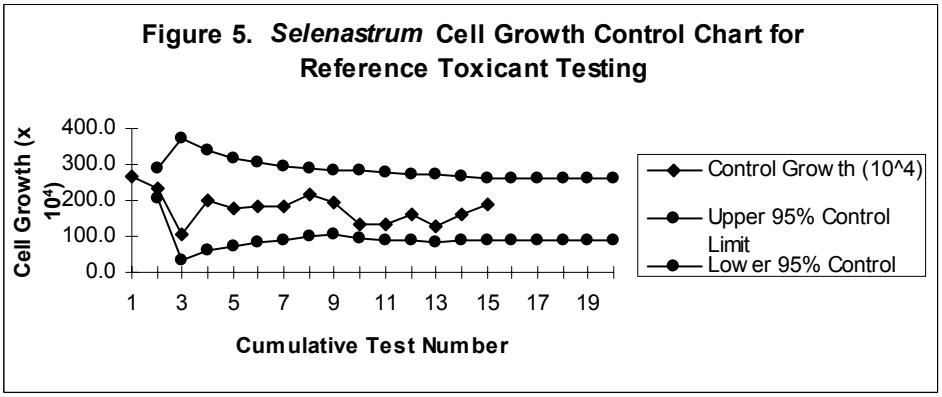



**Figure A5. Control chart for *S. capricornutum* growth***




* Control chart for *S. capricornutum* tests using EDTA

**Figure A6. Control chart for *H. azteca* survival**