QUALITY ASSURANCE PROJECT PLAN

TMDL Development for the Susan River

May 2003 - June 2004

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Prepared for:
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APPROVALS:

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Peter Green; University of California, Davis – Chemical Analysis	Date
Nancy Richard; State Water Resources Control Board - Contract Manager	Date
William Ray; State Water Resources Control Board - Quality Assurance Officer	Date

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1. GENERAL INTRODUCTION

The University of California, Davis Aquatic Toxicology Laboratory (UCD ATL) is a State Certified Laboratory whose primary purpose is to conduct toxicity tests evaluating water quality and aquatic ecosystem health. US EPA (1994 and 2002) toxicity testing methods and Toxicity Identification Evaluation (TIE) methods (1991a, 1993a, and 1993b) as well as other non-EPA methods are used to characterize and identify potential contaminants in aquatic samples. The quality of the data generated at UCD ATL is ensured and implemented through a variety of protocols and criteria established by US EPA and/or UCD ATL. These include, but are not limited to, extensive documentation, as well as implementation of preventative and corrective measures to meet quality assurance objectives.

2. OBJECTIVES OF QUALITY ASSURANCE PROJECT PLAN

The Quality Assurance Project Plan (QAPP) document defines procedures and criteria that will be used for projects conducted by UCD ATL in association with a Contractor. Among other things, criteria for data quality acceptability, procedures for sampling, testing and calibration, as well as preventive and corrective measures are included in this document. The responsibilities of UCD ATL and the SWRCB Contract Manager also are contained herein.

An approved QAPP is required prior to the initiation of any toxicity testing. The Contractor is responsible for submitting a project description that includes a project overview and its goals, as well as submitting a list of sampling sites, the rationale for site section and sampling frequency to UCD ATL.

3. PROJECT DESCRIPTION

The Susan River originates from Silver and Caribou Lakes, in southern Lassen County, and flows east through McCoy Flat Reservoir discharging into Honey Lake. The surrounding areas encompass an abandoned railroad and private mines. Fishing, cycling, hiking, horseback riding, and skiing are popular uses along the Susan River (Friends of the River). The State Water Resources Control Board (SWRCB) and Regional Water Quality Control Board have conducted investigations of California's inland waters over the past twelve years and found toxicity to aquatic organisms. Agriculture, mining, and storm water runoff were revealed to be the primary

contributors to this toxicity. In the early 1990's the US EPA found toxicity in the lower part of the Susan River watershed. In 1996, the Susan River was placed on the Federal Clean Water Act Section 303(d) list of impaired waterbodies for unknown toxicity. This study will investigate the validity of previous sampling studies and identify specific cause(s) of contamination in the Susan River as part of the Lahontan Regional Board's development of a Total Maximum Daily Load (TMDL).

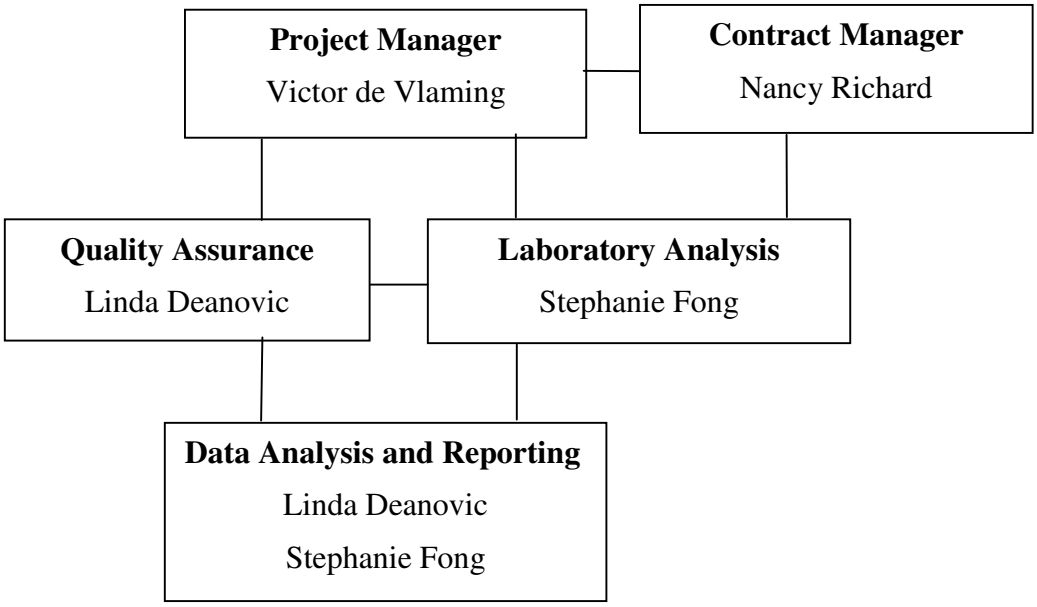
Study objectives:

Conduct toxicity tests, TIEs, and chemical analyses on larval fathead minnows, duckweed, and *Ceriodaphnia dubia* to:

1. Investigate the validity of previous toxicity studies on the Susan River to aid the Lahontan Regional Board in ultimately confirming or denying the need for its placement on the Clean Water Act Section 303(d) list.
2. Identify specific cause(s) and source(s) of toxic contaminants to aid the Lahontan Regional Board in development of a TMDL for toxicity in the Susan River.

4. PROJECT ORGANIZATION AND RESPONSIBILITIES

Figure 1. Summary diagram: lines of communication.



RESPONSIBILITIES	PERSON
Sampling:	
Sampling design	Nancy Richard; SWRCB, Victor de Vlaming; UC Davis, Anne Sutherland, Lahontan RWQCB
Sample collection, calibration of field instruments, field analysis	Nancy Richard; SWRCB, Anne Sutherland; Lahontan RWQCB
Sample delivery	Nancy Richard; SWRCB
Sample storage and custody and lab instrument calibration	Laboratory assistants; UC Davis
Toxicity Testing:	
Toxicity testing, QA/QC, data validation, audits, and corrective actions	Stephanie Fong, Linda Deanovic; UC Davis
Chemical Analyses Quality Control:	
Metals	Tom Young , Peter Green; UC Davis
Pesticides	Tom Young , Peter Green; UC Davis
Chemical Analyses Data Validation:	
Metals	Tom Young , Peter Green; UC Davis
Pesticides	Tom Young , Peter Green; UC Davis
Project Direction:	Victor de Vlaming; UC Davis, Nancy Richard; SWRCB
Project Quality Assurance	Linda Deanovic; UC Davis
Contract Management:	Nancy Richard; SWRCB
Statistical Guidance:	Neil Willits; UC Davis
Data Management and Reporting:	Linda Deanovic, Stephanie Fong; UC Davis

5. SAMPLING PROCEDURES

Sites and Sampling Schedule:

Site locations are based on historical toxicity data, land use practices, accessibility, and runoff patterns. The Lahontan Regional Board staff will collect samples from 4 sites over 8 to 10 sampling events from May 2003 through April 2004. Samples will be collected from the Susan River near the United States Geological Survey (USGS) gage at the Hobo Camp trailhead, at McGowan Lane, Leavitt Lane Bridge, and upstream of Litchfield at Bridge 7-34 on Highway 395. Sample sites and rationale for choosing these sites are listed in Table 1.

Table 1. Summary of site selection criteria.

Site	**Map ID**[1]	**Rationale for Selection**
Susan River near USGS gage at Hobo Camp trailhead to Bizz Johnson trail	SR-1	To duplicate 1990 US EPA toxicity testing site R-6-1, and represent water quality upstream of the City of Susanville.
Susan River at McGowan Lane	SR-2	To capture changes in water quality below confluence with Gold Run Creek, which may have geothermal discharges that could influence water quality. Also near 1990 US EPA site R-6-2.
Susan River at Leavitt Lane Bridge	SR-3	Best available access downstream of confluence with Jensen and Brockman Sloughs where Susanville Consolidated Sanitary District discharges and agricultural activity may influence water quality.
Susan River upstream of Litchfield at Bridge 7-34 on Highway 395	SR-4	To duplicate 1990 US EPA site R-6-3 downstream of confluence with Willow Creek

1. Map IDs refer to sites on Figure 1.

Sample frequency will be approximately monthly, with specific dates agreed upon by UCD ATL and Regional Board staff. If agreed upon by the SRWCB Contract Manager and UCD ATL Director, additional samples may be collected during periods of interest (storm event, etc.). Samplers will collect a minimum of 6 gallons per site. The sample volume collected from each site may vary in months when quality assurance samples are to be tested. The volume of sample, including quality assurance samples, is indicated in Table 2.

Sample Collection:

UCD ATL will provide Lahontan Regional Board staff with pre-cleaned gallon glass amber bottles to collect samples as described in UCD ATL Standard Operating Procedures Manual (SOP), SOP 5-1. In side-by-side tests between glass and plastic containers, UCD ATL found that toxicity due to a non-polar organic chemical was removed by the plastic containers. Amber glass also minimizes photo-degradation of the sample. For these reasons, UCD ATL believes that glass containers preserve sample integrity better than plastic. Although volatilization due to headspace is an issue, we believe that glass amber bottles are the most suitable choice for this study. Sample containers will be rinsed three times with site water prior to sample collection. Samples will be collected from mid-channel, as subsurface grabs off a bridge and placed in wet ice immediately after collection. Where mid-channel samples cannot be collected, shore samples will be collected from a well-mixed portion of the watercourse. During events where a trip blank is called for, UCD ATL will provide Regional Board staff with water to be taken to a specified site that is unopened and then brought back with the other samples.

Sample containers will be labeled with site identification and collection date. The sampling team will record relevant information in the field log book and on the chain of custody (COC) form including: (1) sample identification (a unique number for each sample site), (2) sample location, (3) date and time of sample collection, (4) sampler's name, (5) field instrument readings [including water temperature, pH, dissolved oxygen (DO), and electrical conductivity (EC)], (6) sampling conditions, and (7) deviations (see Figure 1 in the Appendix).
Toxicity test water renewals will be from the initial grab sample. Using a single grab sample for toxicity test renewals facilitates determination of the cause(s) of toxicity.

Table 2. Number of gallons to be sampled at each site (including quality assurance samples).

	Sampling sites			
Event	**SR-1**	**SR-2**	**SR-3**	**SR-4**
1 (May 2003)	7	7	7	7
2 (June 2003)	9[1]	7	7	7
3 (July 2003)	7	7[2]	7	7
4 (August 2003)	7	7	7	7
5 (September 2003)	7	7	9[1]	7
6 (October 2003)	7	7	7	7
7 (T.B.D.[3])	7	7	7	7
8 (T.B.D.[3])	7	7	7	7
9 (March 2004)	7	7	7	9[1]
10 (April 2004)	7	7	7	7
11 (May 2004)[4]	7[2]	7	7	7
10 (June 2004)[4]	7	7	7	7

1. 9 gallons includes extra water for the field duplicate.
2. A laboratory control duplicate (trip blank) will be tested during this month. UCD ATL will send control water to the Lahontan Regional Board prior to sampling and the Regional Board will "sample" this control water at the chosen site.
3. To be determined. Sampling events reserved for re-sampling due to toxicity or additional QA.
4. To replace failed tests.

Sample Transport and Storage:

Samples will be hand-delivered to UCD. Samples bottles will be packed in ice chests with sufficient wet ice to maintain temperatures between 0-10°C and enough packing material to minimize bottle breakage. Upon arrival UCD ATL, sample temperature will be measured. US EPA recommends the temperature at sample receipt to be 0-4°C, but this is not always possible

due to driving distance from sampling sites to laboratory, water temperature at sample collection, and/or ambient temperature. If sample arrival temperature is between 4-10°C, or if ice had formed, the sample will be tested, the data results will be flagged, and the SWRCB Contract Manager notified. If sample arrival temperature exceeds 10°C, the SWRCB Contract Manager will be notified before test initiation. Samples will be stored at UCD ATL in a dark 4±3°C environmental chamber. US EPA recommends sample storage to be at 4±1°C, but not all ATL storage chambers are capable of holding this temperature range. Toxicity tests will be initiated within 48 hours of sample collection.

6. TOXICITY TESTING

General Procedures:

Toxicity testing for *Ceriodaphnia dubia* (a cladoceran, zooplankton species) and larval *Pimephales promelas* (a cyprinid minnow) will follow the Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms (US EPA, 2002). Aspects of these procedures that differ from the US EPA methods, and the rationale for using them, are outlined below. Toxicity testing for *Lemna minor* (a free-floating aquatic plant, duckweed) will follow the American Society for Testing and Materials (ASTM) Standard Guide for Conducting Static Toxicity Tests with *Lemna gibba* G3 (1998).

While US EPA methods do not specifically recommend aeration of the renewal water, the UCD ATL protocols include aeration. This deviation is employed because the ambient samples tested at UCD ATL frequently require aeration to prevent oxygen super-saturation. Aeration time will be limited until sample comes to 102% saturation to minimize the loss of volatile toxicants.

The UCD ATL uses control waters made per UCD ATL SOP 7-1 through 7-4. Sierra Springs™ water amended to EPA moderately hard (SSEPAMH) is used as the control water for the *Ceriodaphnia dubia* test. Deionized water amended to EPA moderately hard (DIEPAMH) is used as the control for the minnow test. Sierra Springs™ water amended with ASTM standard growth media is used as the control water for the duckweed test.

Ceriodaphnia dubia: Cultures originally obtained from Aquatic Research Organisms, New Hampshire, are maintained at UCD ATL (SOP 2-4 and 3-

1). Test organisms employed are less than 24 hours old born within a 16-hour period and derived asexually.

The *Ceriodaphnia dubia* chronic-style test consists of 10 replicate 20ml glass vials each containing one *Ceriodaphnia*. US EPA (1994, 2002) suggests usage of plastic cups, but the UCD ATL opts to use glass vials to minimize chemical sorbtion. *Ceriodaphnia* are transferred into a new vial of fresh test solution with *Selenastrum* and YCT (a mixture of yeast, organic alfalfa, and trout chow) daily. Tests are conducted at 25 ± 2°C with a 16-hour light: 8-hour dark photoperiod. US EPA recommends 25+1°C, but this small range is not always possible at the ATL particularly during winter and summer months. Mortality and reproduction are assessed daily and at test termination. Test parameters are summarized in Table A of the Appendix.

The *Ceriodaphnia dubia* acute Toxicity Identification Evaluation (TIE) tests (US EPA 1991a) consist of four replicate glass vials containing 15 ml of sample with five organisms each. Tests are initiated with less than 24-hour-old *Ceriodaphnia,* born within a 20-hour period. *Ceriodaphnia* are fed a mixture of *Selenastrum* and YCT before test initiation and four hours prior to test renewal. No food is added to the daily renewal waters to minimize toxicant sorption to food particles. *Ceriodaphnia* are transferred into a new vial of fresh test solution daily. Tests are conducted at 25 ± 2°C with a 16-hour light: 8-hour dark photoperiod. Mortality is assessed daily and at test termination. Test parameters are summarized in Table B of the Appendix.

***Pimephales promelas**:* Larvae, hatched in transport, are obtained from AquaTox, Inc. Arkansas (SOP 2-4). When the larvae arrive, they are acclimated with DIEPAMH which is then placed into a 25°C bath and slow, constant aeration is applied. Testing is initiated after acclimation and before the larvae are more than 48 hours old.

The larval *Pimephales promelas* chronic tests consist of four replicate 600 ml Teflon™ beakers, each containing 250 ml of sample and 10 minnows. Less than 48-hour-old minnows, born within a 24-hour period are employed. Minnows are fed before test initiation and three times daily during testing with brine shrimp *Artemia* nauplii. US EPA recommends using glass beakers, but Teflon™ beakers will be used for this project to decrease chances of

bacterial or fungal infection. US EPA suggests feeding twice daily. UCD ATL feeds half the US EPA suggested daily amount each morning and ¼ the recommended daily amount each afternoon and before close of laboratory to reduce bacterial growth in test chambers. Approximately 80 % of the test water is renewed daily. Test water is incubated in a water bath at 25 ± 2°C under ambient laboratory light with a 16-hour light: 8-hour dark photoperiod for seven days. Mortality is measured daily at the time of water renewal and at test termination. At test termination, minnows are euthanized and dried to constant weight. Minnows are then weighed and biomass (growth) is measured. Test parameters are summarized in Table C of the Appendix.

The larval *Pimephales promelas* 96-hour TIE tests consist of four replicate 600 ml Teflon™ beakers, each containing 250 ml of sample and 10 minnows. Less than 48-hour-old minnows, born within a 24-hour period are employed. Minnows are fed before test initiation and twice daily while on test with brine shrimp *Artemia* nauplii. US EPA (1991a) suggests water renewal at 48-hours and a single feeding at 48-hours. Due to the potential for rapid contaminant degradation, sample waters are renewed daily to ensure a more consistent toxicant concentration. UCD ATL feeds half the US EPA suggested amount twice daily to reduce bacterial growth in test chambers. Approximately 80 % of the test water is renewed daily. Test water is incubated in a water bath at 25 ± 2°C under ambient laboratory light with a 16-hour light: 8-hour dark photoperiod for four days. Mortality is measured daily at the time of water renewal and at test termination. Test parameters are summarized in Table D of the Appendix.

***Lemna minor**:* Cultures originally obtained from Carolina Biological Supply, Berlington, NC, maintained at UCD ATL. Test organisms were shipped moist and cultured in-house for three weeks before initial use.

The duckweed 7-day static non-renewal tests consist of no fewer than two replicate 250ml glass beakers, each containing 100ml sample and 12 duckweed fronds comprised of 3-frond colonies. To increase statistical power, and barring a lack of appropriate plants, UCD ATL will use four replicates in these tests. Test water is incubated in an environmental chamber at 25 $\pm$ 2ºC under constant cool-white fluorescent light. Frond growth is measured at test termination. At test

termination, duckweed are dried to constant weight and weighed. Test parameters are summarized in Table E of the Appendix.

Data Management{ TC "DATA MANAGEMENT" \f C \l "2" }

Reduction and Storage - All raw toxicity test, TIE, and sample water quality data will be recorded in non-erasable ink on standardized printed data sheets. The raw data are entered into spreadsheets and manipulated with statistical programs, then photocopied and used when performing data interpretations. All data will be submitted to the SWRCB Contract Manager as part of the corresponding project reports. Summary tables will be generated for the toxicity tests, TIEs, and the water quality parameters. All tables and statistical analyses will be proofread and checked for quality assurance. All data will be filed and stored on site in a secure cabinet for seven years.

Statistical Analysis - Each sample will be characterized by descriptive statistics indicating the mean response and variation among replicates.

Toxicity is defined as a statistically significant mortality difference ($p<0.05$) in an ambient sample compared to laboratory control(s). Specifically, acute toxicity in the *Ceriodaphnia* and larval *Pimephales* assays is defined as statistically significant mortality within 96 hours in a test sample compared to the laboratory control. When toxicity is detected, the SWRCB Contract Manager will be notified as soon as possible.

All *Ceriodaphnia* reproduction, larval *Pimephales* growth and mortality and duckweed growth data will be analyzed with Shapiro-Wilks Test for normality and Bartlett's Test for homogeneity of variance. When data fit normal distributions and have homogeneous variances, they will be analyzed using an Analysis of Variance and Dunnett's mean separation tests. When data deviate significantly from normality or have heterogeneous variances, they will be log transformed. When log transformation does not establish normality or homogeneity of variance, nonparametric Bonferroni corrected Wilcoxan tests will be performed to compare each treatment to the control. *Ceriodaphnia* mortality will be analyzed with Fisher's Exact Test.

These statistical analyses differ from those outlined in US EPA (2002). US EPA statistical procedures were designed for whole effluent toxicity testing in which all samples are tested in a dilution series. The approach to be taken during this study will be to assess water quality at

particular sites compared to laboratory control water. Because these tests will not include a dilution series, the US EPA statistical protocols are not appropriate for the data obtained during this study. ATL staff consulted the UC Davis statistician Neil Willits to determine the most appropriate statistical analyses for these data. The statistician recommended the analyses discussed above.

Quality Assurance{ TC "Quality Assurance" \f C \l "2" }

Quality assurance measures will be included in this project to ascertain the reliability of data gathered including whether UCD ATL testing can be duplicated and to assess whether test species are responding typically, relative to historical test results at UCD ATL. To assess repeatability (precision), laboratory control trip blanks and field duplicates will be tested. To determine whether test species are responding typically during this study, reference toxicant tests will be conducted. The various components of QA activities are summarized below.

Positive control tests- At least one positive control (i.e., reference toxicant) test will be performed monthly. NaCl will be the reference toxicant used for *Ceriodaphnia* and larval fathead minnows and atrazine will be used for duckweed. Reference toxicant tests determine test species sensitivity to a toxicant and whether the test species is reacting typically (within a predetermined range) to that toxicant. These tests will include a laboratory control and a toxicant dilution series in laboratory control water. The LC_{50}/EC_{25} for each reference toxicant test is compared to the UCD ATL running mean to ascertain whether it falls within the acceptable range. The US EPA acceptable range is plus or minus two standard deviations around a running mean. For this project, if a reference toxicant test result does not fall within this acceptable range, results of associated toxicity tests will be considered suspect and identified in interim and final reports. Because the UCD ATL is initiating *Lemna minor* testing for this project, there is no historical data. The running mean will consist of all available data points.

Test acceptability criteria- Test acceptability criteria for chronic *Ceriodaphnia* tests require 80% or greater survival in the controls and 60% or greater of the surviving females must each have a minimum of 15 neonates. Test acceptability criteria for the chronic larval fathead minnow tests require 80% or greater survival in the controls and each minnow must have an average weight of 0.25mg. Test acceptability criteria for 7-day duckweed tests require a five-fold increase in the number of fronds in the controls. When the control performance does not

meet test acceptability criteria, all data from the test are evaluated and noted in interim and final reports. The percentage of chronic *Ceriodaphnia dubia* and larval *Pimephales promelas* tests in which test species control performance met test acceptability criteria at the UCD ATL was evaluated using data from 40 randomly selected tests (per test species) conducted from January 1999 through January 2001. Meeting test acceptability rates were (n=40): 97.5% for *Ceriodaphnia dubia* tests and 92.5% for larval *Pimephales promelas* tests. For *Ceriodaphnia* 96-hours tests, 100% of the tests met the acceptability criteria (n=24).

Deviations and corrective actions- Tests are conducted according to test conditions recommended by the US EPA (2002) with the exception of those reported herein. Beyond those identified herein, deviations from these recommended conditions are reported to the UCD ATL QA Officer. The laboratory director and SWRCB Contract Manager will be notified, as soon as possible within 72 hours of these deviations.

Failure to meet QA criteria can have several outcomes. In some cases, corrective action can occur and in other cases it cannot. For example, if test acceptability criteria are not met with a sample, corrective action will be a re-test of the sample. If samples arrive at the UCD ATL at >10°C or if testing cannot be initiated within the 48 hour maximum sample holding time, the fate of those samples will be determined by the laboratory director on a case by case basis. In the event of standard operating procedure (SOP) deviations, a deviation form will be prepared and the SWRCB Contract Manager notified. UCD ATL SOP references are summarized in Table F of the Appendix.

Best professional judgment will be used in interpretation of results obtained when deviations in the test conditions have occurred. All deviations and associated interpretations will be reported in interim and final reports.

Precision- Precision is the degree to which independent analyses of a given sample agree with one another; it is the reproducibility, consistency, and repeatability of results. Though precision criteria have not been developed for these toxicity tests, UCD ATL assesses precision through several practices that include field duplicates. A field duplicate is a second sample collected in a separate container, immediately after the initial/primary test sample. Test organisms are expected to perform similarly between the sample and its duplicate. Toxicity testing endpoints for field duplicates also have been evaluated to determine the frequency that the UCD ATL data show equivalent results. Paired duplicates were statistically

compared to determine equivalent results. Results can agree (both non-toxic or both toxic) or disagree (one toxic and the other non-toxic).

Table 3 illustrates the frequency that field duplicates in toxicity tests were in agreement (data collected between July 1999 and November 2002). These data demonstrate that there is a high degree of toxicity testing precision at the UCD ATL. Over the last eight years, toxicity test false positives at the UCD ATL have been very infrequent, as demonstrated by re-test, TIEs, and chemical analyses. In samples identified as toxic in initial tests, less than two percent were possibly false positives.

Table 3. Frequency of field duplicates sharing equivalent results.

Test Parameter	Sample Size (n)	Duplicates in Agreement (%)
Ceriodaphnia Mortality (7-day test)	23	95.7
Ceriodaphnia Mortality (96-hour test)	5	100.0
Larval *Pimephales* Mortality (7-day test)	20	100.0
Lemna minor (not yet tested)	NA	NA

In this project, duplicates will be compared by statistical analysis to assess differences. If statistical differences ($p<0.05$) are observed between duplicates the original data will be considered suspect. Results of these analyses will be presented in interim and final reports. The relative percent difference (100x{ |Duplicate 1 - Duplicate 2| / [(Duplicate 1 + Duplicate 2)/2]}) between field duplicates at the UCD ATL has been calculated for several *Ceriodaphnia dubia* and larval *Pimephales promelas* toxicity testing and water quality parameters (Table 4).

Table 4. Summary of laboratory precision at the UCD ATL (July 1999-November 2002).

Test Parameter	Sample Size (n)	Average % Difference	Standard Error
Hardness	28	10.6	2.6
Alkalinity	28	8.2	2.3
pH	29	1.6	0.4
EC	29	6.6	1.7
Ammonia	27	19.0	10.3
Chronic *Ceriodaphnia* Mortality	25	2.7	3.6
Chronic *Ceriodaphnia* Reproduction	22	4.5	2.4
Chronic larval *Pimephales* Biomass	22	15.7	10.2
Chronic larval *Pimephales* Mortality	22	16.1	10.71
Lemna minor (not yet tested)	NA	NA	NA

Chemical analysis- With each chemical analysis, samples are split into autosampler vials. Recovery for OP standards is 116%, with a standard devation of 23%. Using the gas chromatograph mass spectrometer (GC-MS), chromatographs were obtained for samples, blanks and controls, as well as two sets of standards.

7. WATER QUALITY

Various water quality parameters other than contaminants can affect toxicity test results. Thus, UCD ATL monitors several factors that could confound test results to aid in toxicity data interpretation. Water quality parameters of temperature, electrical conductivity (EC), pH, and dissolved oxygen (DO) are measured on all samples at test initiation; temperature, pH and DO are measured at the 24-hour sample renewal. Laboratory pH is measured with a Beckman IS 425 pH meter, DO is measured with a YSI model 58 oxygen meter with a 5700 series probe, and EC is measured with a YSI model 33 EC meter. All meters are calibrated daily according to the manufacturers' instructions. Ammonium is measured on all samples within

48 hours of receipt with an Aquaquant® ammonium kit (EM Science). Unionized ammonia is calculated using the formula in US EPA Update of Ambient Water Quality Criteria for Ammonia (1998). Hardness and alkalinity are measured on all samples within 10 days of receipt, utilizing titrimetric methods. Turbidity is measured within 10 days of receipt with a HACH 2100A Turbidity meter. Instrument calibration and preventative maintenance are summarized in Section 16.

8. CONSIDERATIONS AND CONSTRAINTS

US EPA recommends that toxicity tests be initiated within 36 hours of sample collection. The UCD ATL makes every effort possible to initiate tests within 36 hours of sample collection. If the UCD ATL is unable to initiate toxicity tests within 48 hours, the SWRCB Contract Manager will be notified immediately. Although storage at 4 ± 2°C in darkness generally slows or inhibits degradation of toxicants, increased holding times can result in reduced concentration(s) of some sample contaminants. Degradation and/or adsorption of toxicants on container surfaces during the holding period also can result in underestimation of toxicity and yield false negatives. Sampling will be timed to minimize holding time. Results of tests where samples were held more than 48 hours prior to test initiation will be specifically identified in interim and final reports.

9. REPRESENTATIVENESS{ TC "REPRESENTATIVENESS" \F C \L "2" }

Representativeness refers to the degree to which data accurately represent responses of resident populations at the site where the sample was collected. Estimating risk to indigenous aquatic biota using ambient sample toxicity involves estimation of magnitude, duration of exposure, and the geographic extent of the toxicity. Most UCD ATL projects are intended to measure toxicity and estimate adverse impacts to resident aquatic ecosystem biota.
The US EPA Technical Support Document (1991b) summarizes several studies that support the use of EPA's three freshwater chronic toxicity protocols. These species are generally considered appropriate surrogates (indicator species) for indigenous freshwater biota.
Toxicity test results will be considered representative of toxicity at the sampling site if the sampling protocol is followed, tests are initiated within the holding time and laboratory water

chemistry results are within ranges observed in the field. Recent review articles conclude that US EPA toxicity test results are effective predictors of impacts to resident biota (Waller *et al.*, 1996; de Vlaming and Norberg-King, 1999). Thus, the UCD ATL considers toxicity test results to be indicative of resident species responses when appropriate evaluation of field exposure is included.

10. COMPLETENESS

Completeness is a measure of the data obtained compared to the amount of data expected in a project. The toxicity data acquisition phase of a project is considered complete when all sites specified in a contract have been visited the number of times designated in that contract, the number of samples designated in the contract have been collected and the number of toxicity tests and TIEs designated in the contract have been successfully completed (as described in other sections of this document).

Most UCD ATL projects are intended to provide an assessment of surface water toxicity and an identification of its cause(s) in a particular watershed or subsection thereof. UCD ATL will provide the SWRCB Contract Manager with quarterly reports of data results in tabular form. An interpretive report including a prediction of potential impacts of toxicity to aquatic ecosystem biota in the Susan River watershed will be provided to the SWRCB Contract Manager at the termination of this project. Such predictions are restricted to the spatial and temporal scale of the project and are therefore, not intended to be a complete characterization of the watershed. Uncertainty is associated with all biological data but that can be decreased and completeness enhanced with a larger number of sampling sites, an increased frequency of sampling/testing, duration of study and inclusion of additional monitoring and assessment procedures (i.e., bioassessments, chemical analyses, *in situ* testing and etc.).

11. COMPARABILITY{ TC "COMPARABILITY" \F C \L "2" }

Comparability relates to similarity of data from different data sets and sources; it is an indication of the confidence with which one data set can be compared to another. With the exceptions noted herein, the UCD ATL strictly documents and adheres to US EPA test protocols, UCD ATL SOP's, QA measures outlined herein, and acceptable reference toxicant test results. Therefore, the laboratory results obtained in one project can be compared to

results from previous UCD ATL projects as well as from other laboratories using the US EPA procedures.

12. TEST SENSITIVITY{ TC "TEST SENSITIVITY" \F C \L "2" }

Test sensitivity refers to the ability to distinguish a statistical difference between test organism response in laboratory control water compared to an environmental sample. Test sensitivity is frequently expressed as the percent difference between the control and environmental sample that can be detected. The level of effect that can be detected will vary, depending on control performance, variability among replicates, the test species, and endpoint measured. UCD ATL typically has been able to detect approximately 20% or more difference from controls. At this time, UCD ATL does not have acceptability criteria for test sensitivity. The lower the test sensitivity, the greater the probability of false negatives (sample is toxic but test does not detect toxicity). Test sensitivity can be increased by increasing the number of replicates. That, in turn increases the costs of testing. UCD ATL will identify test results in which the ability to distinguish a difference between control and ambient water sample was 30% or greater.

13. DATA AUDITS{ TC "DATA AUDITS" \F C \L "2" }

All data reported for this project will be subject to a 100% check for errors in transcription, calculation, and computer input by the UCD ATL QA Officer. Additionally, the QA Officer will review all sample logs and data forms to ensure that requirements for sample holding times, sample preservation, sample integrity, data quality assessments, and equipment calibration have been met. At the discretion of the Laboratory Director, data that do not meet these requirements will either not be reported or will be reported with an explanation of associated problems.

14. CORRECTIVE ACTION

Depending on the parameter, failure to meet QA criteria can have several outcomes. In some cases, corrective action can occur and in other cases it cannot. For example, if toxicity test acceptability criteria was not met with a sample, corrective action could be a re-test of the sample or substitution of a sample(s) collected from the same site(s) at a later date. On the other

hand, if samples arrive at UCD ATL exceeding temperatures previously specified, the SWRCB Contract Manager will be consulted to determine whether or not those samples will be tested. If testing cannot be initiated within the maximum sample holding time designated, the SWRCB Contract Manager will be contacted to determine proceedings. In such cases, the SWRCB Contract Manager may decide to extend the holding time or request a substitution of sample(s) collected from the same site(s) at a later date. In such cases, corrective action would be an alteration of procedures that ensure the arrival of future samples to achieve the specified temperature and sample holding times.

In the event of SOP/QAPP deviations, a deviation form will be written and the SWRCB Contract Manager will be notified. Protocol Amendments will be employed when the procedure changes from the standard protocols. Corrective action, SOP/QAPP deviations, and Protocol Amendments are described in the SOP Manual. The specific procedures are referenced in the Appendix.

15. PERFORMANCE AND SYSTEM AUDITS{ TC "PERFORMANCE AND SYSTEM AUDIT" \F C \L "2" }

The SWRCB Contract Manager or their designee may conduct inspections of the physical facilities, operational systems, and operating procedures at UCD ATL. The inspections can be conducted while toxicity tests are being performed; the facility should be given 24-hour notice of the inspections.

16. TOXICITY IDENTIFICATION EVALUATIONS (TIES)

Background

The next step in toxicity testing is to determine the cause(s) of toxicity. That is, mitigation activities, be they volunteer or regulatory based, are greatly facilitated when the cause(s) of toxicity is/are known. Thus, a major effort can be made to specifically identify the cause(s) of toxicity in toxic samples. Toxicity Identification Evaluations (TIEs) consist of physical, chemical, and toxicological manipulations designed to identify the specific toxicant or class of chemicals responsible for toxicity observed in a sample (US EPA, 1991a). TIEs will be performed on *Ceriodaphnia* or larval *Pimephales* that exhibit 50% or greater mortality and statistical differences from the control within 96 hours in the initial test. Test acceptability for

all *Ceriodaphnia* and larval *Pimephales* 96-hour tests requires 90% or greater survival in the controls. Abbreviated TIEs will be performed on duckweed that exhibit ≤50% the growth of the controls. Follow-up procedures are summarized in Figures 1 and 2 of the Appendix.

Predicting the number of toxic samples that will be observed in a project is impossible. The number of TIEs conducted by UCD ATL will be no more than 6 samples due to budgetary constraints of this contract.

US EPA states that "TIEs require that toxicity be present frequently enough and endure storage … so that repeated testing can characterize … and confirm toxicants," also, "enough testing should be done to assure consistent presence of toxicity before TIEs are initiated (US EPA 1991b). UCD ATL does not always adhere to these recommendations. While some contracts specify re-sampling at a site and testing upon observing toxicity for a pre-determined magnitude, returning to widely dispersed and/or distant sampling sites to determine toxicity persistence often is not affordable. Following rainstorms, pulses of toxicity may endure for hours, days, or over a week. Such data provide information on a point in time, not in persistence of toxicity. Repeated sampling, testing and TIEs at a site are necessary for estimating toxicity duration (persistence) and chemical cause(s). Method blanks are tested concurrently with manipulated sample waters in TIEs to ensure that manipulations do not cause toxicity. Method blanks are compared to the laboratory control, and if they are statistically different from the control, treatments using the questionable manipulation are rejected. Samples exhibiting toxicity in the initial screening are tested concurrently with manipulated samples to confirm original toxicity and provide appropriate statistical comparisons. No follow-up work will be initiated on samples more than 6 weeks past collection date, unless requested by the contract manager.

Phase I TIE procedures provide information on the physical/chemical characteristics and nature of the toxicant(s) in the toxic sample. For instance, is the chemical volatile, chelatable, filterable, reducible, non-polar organic, or pH sensitive?

Phase II TIE work or additional chemical analyses that may be required to strengthen TIE conclusions may be conducted in-house or subcontracted as specialization and workload dictates. The decision to subcontract out the Phase II TIE procedures is generally made jointly by the SWRCB Contract Manager and the UCD ATL.

TIEs include chemical analyses. As indicated, the number of TIEs performed depends on the number of toxic samples and available budget. Likewise, the number of chemical analyses that can be conducted also depends on these factors. The UCD ATL does not perform chemical analyses, such work is subcontracted. Contractors are encouraged to consult with UCD ATL in designating a budget component for TIEs and chemical analyses.

Follow-up on a toxic sample can be initiated at the discretion of the SWRCB Contract Manager. Follow-up may include, but are not limited to:

- re-sampling a toxic site to estimate the duration and frequency of toxicity
- a dilution series test to estimate the magnitude of toxicity
- sampling additional sites to determine the origin/source of toxicity

Dilutions

Generally, point estimations (e.g., LC_x or EC_x) and associated statistics are used for multiple dilution test data. A point estimate is the concentration of a particular toxicant that results in some level of response (e.g., mortality, number of offspring) in the test organisms. For example, an LC_{50} is the concentration of a toxicant that causes mortality in 50% of the test organisms.

Dilution series tests will be performed to determine the magnitude/potency of toxicity in a toxic sample. Results of these tests will be used to estimate the toxic units (TUs) in a toxic sample. Toxic units are estimated by dividing the 100% sample by the lowest sample dilution causing toxicity. For example, if the sample diluted to 25% causes toxicity, the sample consists of at least four 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 LC_{50} concentration for the species of interest. An LC_{50} 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 determined by dilution of the ambient water sample. Dilution series tests are generally performed on samples causing 100% mortality within 24-hours to either *Ceriodaphnia* or larval *Pimephales*. Dilutions will consist of 100, 50, 25, 12.5, and 0% of the sample. Dilutions are made with control water for each respective species.

Phase I TIEs

The purpose of Phase I TIEs is to identify the class(es) of contaminant(s) causing the toxicity. The toxicity tests associated with TIE procedures are performed as described above; additional sample manipulations are performed to reveal the cause(s) of toxicity.

Solid Phase Extraction (SPE) columns remove non-polar organic chemicals from aqueous test samples as it is passed through. Toxic samples are passed through an SPE column and these waters are tested along with the unmanipulated sample. Control water also is passed through an SPE column and serves as one of the method blanks. The adsorbate is then eluted with methanol and the eluate is added to control water and tested along with the appropriate method blanks. If the toxicant is a nonpolar organic chemical, the ambient sample and control water amended with eluate will exhibit high mortality while the sample passed through the SPE column results in reduced or no mortality.

In some cases, binding of metals to organic and inorganic ligands in samples will reduce the bioavailability of metals. The extent of metals binding to organics can be estimated by comparing the toxicity of the sample before and after solvent extraction, since solvent extraction removes organic-bound metals. Disodium Ethylenediamine Tetraacetate (EDTA) and Sodium Thiosulfate (STS) bind to various metals, making them unavailable to biota. Three concentrations of each EDTA and STS will be added separately to toxic samples and tested along with the appropriate controls. If the toxicant is one of these metals, the ambient sample will exhibit high mortality while the ambient sample amended with EDTA or STS results in reduced or no mortality.

Air stripping sometimes reduces or removes surfactants and/or ammonia from waters. Toxic samples will be air stripped and tested along with the appropriate control. If the toxicant is a surfactant, the ambient sample will exhibit high mortality while the air-stripped sample usually results in reduced or no mortality.

Additionally, in the *Ceriodaphnia* Phase I TIE, samples are amended with piperonyl butoxide (PBO). PBO inhibits or reduces toxicity caused by metabolically activated organophosphorous (OP) insecticides such as diazinon, chlorpyrifos and malathion (Bailey *et al.*, 1996). 100 µg/L PBO is added to the toxic samples. The 'original' ambient test sample and the ambient test 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 ambient test

sample will exhibit high *Ceriodaphnia* mortality while the ambient test sample amended with PBO results in reduced or no *Ceriodaphnia* mortality.

TIEs to be conducted on acutely toxic samples will employ protocols outlined in US EPA (1991a, 1993a, 1993b), Bailey *et al*. (1996), Connor and Deanovic (1991), and Deanovic *et al*. (1996 and 1998). The Phase I TIE will include a retest of the toxic sample to confirm toxicity and manipulations that may include, an EDTA series, a C8 solid phase extraction (SPE) column and add-backs, a PBO treatment (with *Ceriodaphnia*), filtration, and aeration, an STS series, other SPE resins, and pH shifts.

Phase II TIEs

The purpose of Phase II TIEs is to identify the constituent(s) causing or contributing to the toxicity. If the Phase I TIE suggests that the toxicity is due to cationic metals (e.g. removal of toxicity by EDTA and STS), the sample will be submitted for metals analysis. If the Phase I TIE suggests toxicity due to non-polar organic constituents, the sample will be concentrated on SPE columns and fractionated. Fractions are added to control water and tested with the appropriate species.

Chemical Analysis

Samples causing 50% or greater decrease in *Lemna minor* growth in the initial screening will be passed through an SPE column. Toxicants adsorbed on the column will be eluted with 100% methanol and submitted along with1-4L of the unmanipulated sample to Peter Green at UCD for chemical analysis through liquid: liquid extraction and processed through the GC-MS. Samples causing toxicity to *Ceriodaphnia dubia* or larval *Pimephales promelas* are re-tested in the Phase I TIE to confirm that toxicity persists after storage and identify the class of toxicant. At this time, the sample may be submitted to Peter Green for chemical analysis. No more than 10 samples will be submitted for metal or organic chemical analyses. Toxicants in the sample can degrade as a result of extended holding time. Contractors should be aware that chemical concentrations may be an underestimation of the concentration in the sample at time of collection and testing. Chemicals causing toxicity have been heavy metals, ammonia and organophosphorous and carbamate pesticides.

17. REPORTING REQUIREMENTS

The following products are to be delivered by the University or its subcontractors to the State Board:

1. Stephanie Fong shall regularly brief the SWRCB Contract Manager on the progress of all on-going toxicity tests, TIEs, and special studies in a timely manner. Any toxicity or mortality will be reported as soon as possible to the SWRCB Contract Manager.

2. Quarterly progress reports describing the work performed, any problems encountered while conducting tests, including an assessment of the effect of these problems on test results, and describe measures taken to correct problems. Ultimately, the cause and source of toxicity will be furnished in a written assessment and reported in the quarterly reports to the SWRCB Contract Manager.

3. A final report will be prepared to include a description of methods, all raw data and associated statistical analysis in tabular form, results of all quality assurance and quality control work, and a discussion of the results and conclusions of the basic monitoring, TIEs, and other special studies. The discussion of the results of this study shall include, where possible, the frequency and level of toxicity in the sampled waters, and identification of the toxicant or class of toxicants associated with observed toxicity, the probable source(s) of toxic chemicals, the ecological effects of toxic run-off to the river, a review of pertinent literature, and a comparison of study results with similar studies performed in California and other parts of the United States. The report will also include recommendations for future work.

18. CALIBRATION PROCEDURES

Laboratory instruments are calibrated, standardized and maintained according to procedures detailed in the SOP Manual. Section 8 of the SOP, “Instrument Protocols”, identifies step-by-step calibration and maintenance procedures. EC and pH meters are checked against known standards every five weeks for precision. Data generated from the quality assurance checks will be incorporated into a control chart. Prior to use, field instruments are calibrated and recorded in the field log book.

- **Mettler AE 100 Balance**: Used for the routine weighing of chemicals. Before operation, the balance is verified to be level. Adjustments are made to level properly if necessary. An internal calibration is performed any time the balance is unplugged or moved. Prior to use the balance is checked with reference weights. The balance is serviced and calibrated by a quality control service annually.
- **Mettler H54AR Balance**: Used for the routine weighing of fish and weigh boats. Before operation, the balance is verified to be level. Prior to use the balance is zeroed and then checked daily with reference weights. The balance is serviced and calibrated by a quality control annually.
- **Max/Min Thermometers**: Used to detect the maximum and minimum fluctuations in temperature over a given time period in environmental chambers, refrigerators and water baths. Mercury thermometers are calibrated using a NIST certified thermometer annually.
- **Model ZM Coulter-Counter**: Used to determine algal growth by counting the number of cells, of a given size in a given volume of fluid. Though the Coulter-Counter is not calibrated a control count is performed on a solution with a known concentration of microspheres (counting beads). The Coulter-Counter is oiled every 5 weeks and the tubing is maintained with isotonic solution detergent.
- **YSI Model 33 Electrical Conductivity (EC) Meter**: Used to determine the electrical conductivity and/or salinity of a water sample. This meter has an internal calibration that is performed daily. The internal cell constant is calibrated every five weeks with a traceable conductivity calibration standard. At this time the probe is also checked and cleaned when there are traces of hard water deposits, oils and organic matter.
- **Beckman 12 pH/ISE Meter**: Used to measure the pH of a water sample. It is calibrated daily against two buffers (7.0 and 10.0). Every six weeks it is checked against a secondary precision pH buffer of 7.0 and 10.0. pH meter probes are checked weekly for algae buildup and for appropriate fluid levels. pH buffers and KCL storage solutions are changed every five5 weeks.
- **YSI Dissolved Oxygen (DO) Meter 58**: Used to determine the concentration of dissolved oxygen in a water sample. The probe is daily zeroed and calibrated in

saturated, pure water at test temperature. The probes are checked every five weeks for bubbles and wrinkles and the membrane is replaced, if necessary.

- **HACH Model 2100A Turbidimeter**: Used to determine Nephelometric Turbidity Units (NTUs) of an ambient sample. The meter is daily calibrated with NTU standards that are within the range for the water sample.
- **EM Science Aquaquant Ammonium kit**: Used to determine ammonia content of a sample. A standard and a blank are run to ensure the reagents are reacting properly.

19. LABORATORY ORGANIZATION AND RESPONSIBILITY

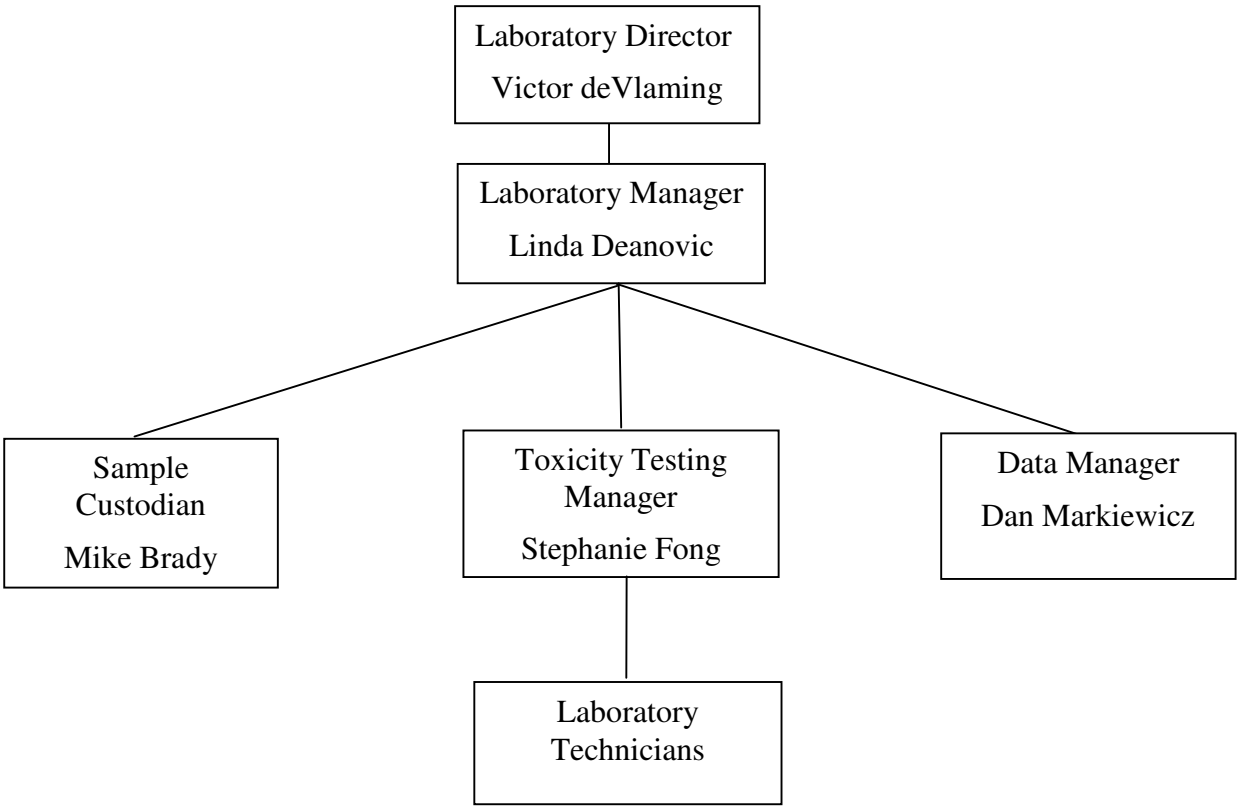


Table 4. Positions and Duties

POSITION	PERSON	RESPONSIBILITIES
Director	Dr. Victor deVlaming	Overall direction of the laboratory's research
Manager	Linda Deanovic	Organizing, coordinating, planning and designing research projects and supervising laboratory staff.
Sample Custodian	Mike Brady	Sample design, sampling coordination and operations, sample storage and disposal.
Toxicity Testing Manager	Stephanie Fong	Direct communication with contract managers and clients in all projects and communicating any client challenges and concern to the Director, Manager, Sample Custodian and/or Data Manager in order to resolve any issues.
Data Manager	Dan Markiewicz	Statistical Analysis, generating of summary tables to the client upon request.
Technicians	Additional Staff	Conduct toxicity tests, TIEs and measure water quality parameters.

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APPENDIX

Table A. Summary of Toxicity Test Conditions for the Chronic *Ceriodaphnia dubia* Survival and Reproduction Test

1. Protocol	US EPA 1994 and 2002
2. Species	*Ceriodaphnia dubia*
3. Age	Less than 24 hours old and all born within an 8 or 16 hour period
4. Test type	Static renewal
5. Test duration	6-8 days (60% of control females have 3 broods)
6. Endpoints	Survival and reproduction
7. Temperature	25 ± 2°C
8. Photoperiod	16 hours light and 8 hours dark
9. Test chamber size	20 ml scintillation vials
10. Test solution volume	15 ml
11. Renewal of test solution	Daily, 100% renewal
12. Number of neonates/test chamber	1
13. Number of replicates/sample	10
14. Feeding	YCT and *Selenastrum*, See SOP 1-2, 2-1, 9-3, and 9-5.
15. Aeration	Aeration is required only if the DO exceeds the DO tolerances given at 25 ± 2°C, or if the sample DO is below 4 mg/L.
16. Dilution water	Sierra Springs™ water amended to EPA moderately hard.
17. Dilution series (this is not a typical procedure for the UCD ATL testing)	100%, 50%, 25%, 12.5%, 6.25%, 0%. Note that samples will be tested at 100% only (unless 100% mortality is observed within 24 hours or if the sample is for an NPDES permit)
18. Water chemistry	Initially, samples will be tested for DO, temperature, pH, and EC. Water samples will be tested for DO, pH and temperature after 24 hr exposure. Ammonia measurements will be measured on samples within 24 hours of receipt. Alkalinity and hardness will be conducted within 10 days of sample collection
19. Culturing procedures	See SOP 3-1
20. Sample filtration	53 µm plankton net
21. Light quality	Fluorescent with a light diffuser panel
22. Light intensity	50-100 ft-c

Table B. Summary of Toxicity Test Conditions for the Acute *Ceriodaphnia dubia* TIEs

1. Protocol	US EPA 1991
2. Species	*Ceriodaphnia dubia*
3. Age	Less than 24 hours old and all born within a 20-hour period
4. Test type	Static renewal
5. Test duration	4 days
6. Endpoints	Survival
7. Temperature	25 ± 2°C
8. Photoperiod	16 hours light and 8 hours dark
9. Test chamber size	20 ml scintillation vials
10. Test solution volume	15 ml
11. Renewal of test solution	Daily, 100% renewal
12. Number of neonates/test chamber	5
13. Number of replicates/sample	4
14. Feeding	YCT and *Selenastrum*, See SOP 1-7, 2-1, 9-4, and 9-5.
15. Aeration	Aeration is required only if the DO exceeds the DO tolerances given at 25 ± 2°C, or if the sample DO is below 4 mg/L.
16. Dilution water	Sierra Springs™ water amended to EPA moderately hard.
17. Dilution series (this is not a typical procedure for the UCD ATL testing)	100%, 50%, 25%, 12.5%, 6.25%, 0%. Note that samples will be tested at 100% only (unless 100% mortality is observed within 24 hours or if the sample is for an NPDES permit)
18. Water chemistry	Initially, samples will be tested for DO, temperature, pH, and EC. Water samples will be tested for DO, pH and temperature after 24 hr exposure. Ammonia measurements will be measured on samples within 24 hours of receipt. Alkalinity and hardness will be conducted within 10 days of sample collection. If for a TIE, a daily sample DO will be measured and pH will be measured on the 24-hr unmanipulated sample.
19. Culturing procedures	See SOP 3-1
20. Sample filtration	53 µm plankton net
21. Light quality	Fluorescent with a light diffuser panel
22. Light intensity	50-100 ft-c

Table C. Summary of Toxicity Test Conditions for the Chronic Larval Fathead Minnow (*Pimephales promelas*) Survival and Biomass Test

1. Protocol	US EPA 1994 and 2002
2. Species	*Pimephales promelas* larvae
3. Age	Less than 48 hours old
4. Test type	Static renewal
5. Test duration	7 days
6. Endpoints	Survival and biomass (growth)
7. Temperature	25 ± 2°C
8. Photoperiod	16 hours light and 8 hours dark
9. Test chamber size	600 ml Teflon ™ beaker
10. Test solution (volume)	250 ml/replicate
11. Renewal of test solutions	Daily, 80% renewal of original sample
12. Number of larvae/test chamber	10
13. Number of replicates/sample	4
14. Feeding	Artemia nauplii see SOP 1-3
15. Aeration	Aeration is required only if the DO exceeds the tolerances given at 25 ± 2°C or if the sample DO is below 4 mg/L.
16. Dilution water	Deionized water amended to EPA moderately hard
17. Dilution series (this is not a typical procedure for the UCD ATL testing)	100%, 50%, 25%, 12.5%, 6.25%, 0%. Note that samples will be tested at 100% only (unless 100% mortality is observed within 24 hours or if the sample is for an NPDES permit)
18. Water chemistry	Initially, samples will be tested for DO, temperature, pH, and EC. Water samples will be tested for DO, pH and temperature after 24 hr exposure. Ammonia measurements will be measured on samples within 24 hours of receipt. Alkalinity and hardness will be conducted within 10 days of sample collection.
19. Culturing procedures	Received as larvae (SOP 2-4)
20. Sample filtration	53µm plankton net
21. Light quality	Ambient laboratory illumination with light diffuser panel.
22. Light intensity	50-100 ft-c (ambient laboratory levels)
23. Cleaning	Siphon daily with turkey baster immediately before test solution renewal

Table D. Summary of Toxicity Test Conditions for the Acute Larval Fathead Minnow (*Pimephales promelas*) TIEs

1. Protocol	US EPA 1991
2. Species	*Pimephales promelas* larvae
3. Age	Less than 48 hours old
4. Test type	Static renewal
5. Test duration	4 days
6. Endpoints	Survival
7. Temperature	25 ± 2°C
8. Photoperiod	16 hours light and 8 hours dark
9. Test chamber size	600 ml Teflon™ beaker / 150 ml glass beaker
10. Test solution (volume)	250 ml per replicate / 100 ml per replicate
11. Renewal of test solutions	Daily, 80% renewal of original sample
12. Number of larvae/test chamber	10 / 5
13. Number of replicates/sample	4
14. Feeding	Artemia nauplii see SOP 1-6
15. Aeration	Aeration is required only if the DO exceeds the tolerances given at 25 ± 2°C or if the sample DO is below 4 mg/L.
16. Dilution water	Deionized water amended to EPA moderately hard
17. Dilution series (this is not a typical procedure for the UCD ATL testing)	100%, 50%, 25%, 12.5%, 6.25%, 0%. Note that samples will be tested at 100% only (unless 100% mortality is observed within 24 hours or if the sample is for an NPDES permit)
18. Water chemistry	Initially, samples will be tested for DO, temperature, pH, and EC. Water samples will be tested for DO, pH and temperature after 24 hr exposure. Ammonia measurements will be measured on samples within 24 hours of receipt. Alkalinity and hardness will be conducted within 10 days of sample collection.
19. Culturing procedures	Received as larvae (SOP 2-4)
20. Sample filtration	53µm plankton net
21. Light quality	Ambient laboratory illumination with light diffuser panel.
22. Light intensity	50-100 ft-c (ambient laboratory levels)
23. Cleaning	Siphon daily with turkey baster immediately before test solution renewal

Table E. Summary of Recommended Toxicity Test Conditions for the Duckweed (*Lemna minor*) Growth Test

1. Protocol	ASTM
2. Species	*Lemna minor*
3. Species age	2-4 fronds
4. Test type	Static renewal
5. Test duration	7 days
6. Temperature	25 ± 2°C
7. Endpoint	Growth (Frond numbers)
8. Photoperiod	Continuous illumination
9. Test chamber size	250ml Beaker
10. Test solution volume	100 ml/replicate
11. Renewal of test solutions	48-hr intervals; 80% renewal of sample
12. Initial density/test chamber	12 fronds (colonies of 2-4)
13. Number of replicates/sample	4
14. Feeding	2% volume of 100% Growth media made according to ASTM (1998) at initiation and renewal
15. Aeration	None, waters are warmed without aeration to 25±2°C at test initiation
16. Dilution water	Glass distilled water
17. Dilution (this is not a typical procedure for the UCD ATL testing)	Samples will be tested at 100% only
18. Water chemistry	Initially, samples will be tested for DO, temperature, pH, and EC. Water samples will be tested for DO, pH and temperature after first sample renewal. Ammonia measurements will be measured on samples within 24 hours of receipt. Alkalinity and hardness will be conducted within 10 days of sample collection.
19. Culturing procedures	ASTM (1998)
20. Sample filtration	53µm plankton net
21. Light quality	"Cool White" fluorescent lighting
22. Light intensity	400 ± 40 ft-c or as close as possible to this range
23. Randomization	Twice daily

Table F. UCD ATL SOP References for Procedures/Equipment

PROCEDURE/EQUIPMENT	SOP Number
4°C and 25°C Water Baths	8-15
Alkalinity	6-6
Ammonia	6-3, 6-13
Balances	8-2, 8-3
Ceriodaphnia Acute 24-96 hr. toxicity testing, Toxicant Identification Evaluation (acute) for *Ceriodaphnia*	1-7
Ceriodaphnia culturing	3-1
Ceriodaphnia toxicity testing	1-2
Cleaning of Glassware	10-1
Corrective Actions	12-1
Dissolved Oxygen Meter	8-10, 8-11
EC Meter	8-8, 8-16, 8-17
Fathead minnow toxicity testing	1-3
Field Equipment and Sampling	5-1, 5-2, 13.6, 13.7
Metals analysis: Caltest	See Caltest
Pesticide analysis (APPL Inc., GC/MS)	See APPL
Pesticide analysis (in-house ELISA)	6-8, 6-9
pH Meters	8-9, 8-13
Preparation of Food Algae	9-3
Preparation of YCT	9-5
Preservation of samples for pesticide analysis	6-14
Preservation of samples for metals analysis	6-7
Protocol Amendment	12-3
SOP/QAPP Deviation	12-2
Thermometers	8-5, 8-6, 8-12
Total and Calcium Hardness	6-1, 6-2
Toxicant Identification Evaluation (acute) 48-96 hr for fathead minnow	1-8