

QUALITY ASSURANCE PROJECT PLAN

PELAGIC ORGANISM DECLINE

Effects of Toxic Contaminants on Invertebrates and Fish in the
Sacramento-San Joaquin Delta
April 2008- March 2010

Prepared by:

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Draft Version 2.0

Prepared for:

Department of Water Resources

Section A1. Title and Approval Sheets; Citation for QAPP; Preface

Program Title	Effects of Toxic Contaminants on Invertebrates and Fish in the Sacramento-San Joaquin Delta
Performing Laboratory	University of California, Davis Aquatic Toxicology Laboratory 1321 Haring Hall Davis, CA 95616
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Effective Date	This Quality Assurance Project Plan (QAPP) is effective from April 1, 2008 to March 31, 2010 unless otherwise revised, approved and distributed accordingly at an earlier date.
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Preface

This Quality Assurance Project Plan (QAPP) document defines procedures and criteria that will be used for projects conducted by the University of California Davis, Aquatic Toxicology Laboratory (UCD ATL), in association with the Department of Water Resources (DWR). Included are criteria for data acceptability, procedures for sampling, testing (including deviations) and calibration, as well as preventative and corrective measures. The responsibilities of UCD ATL and of the DWR Contractor also are contained within.

The DWR Contractor is responsible for providing a project description that includes a project overview and its goals as well as for submitting a field site list and rationale, and sampling frequency to the UCD ATL Laboratory Manager. The Contractor also determines the sampling sites, number of samples to be collected, and types and number of tests to be conducted.

A QAPP is required for toxicity testing. These QA project plans are drafted and approved by laboratory management prior to test initiation. If, after fully reading this document and becoming knowledgeable of potential deviations, constraints and considerations that must be taken into account, the client wishes to proceed with toxicity testing with UCD ATL, this UCD ATL QAPP will be applied.

Approvals:

Inge Werner, UCD ATL Principal Investigator

_____ Date _____

Linda Deanovic, UCD ATL Laboratory Manager

_____ Date _____

Joy Khamphanh, UCD ATL Toxicity Testing Manager

_____ Date _____

Marie Stillway, UCD ATL Quality Assurance Officer

_____ Date _____

Kevin Reece, DWR Contract Manager

_____ Date _____

Murage Ngatia, DWR Quality Assurance Officer

_____ Date _____

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Section A3. Distribution List and Contact Information

A copy of this QAPP, in hardcopy or electronic format, is to be received and retained by at least one person from each participating entity. At least one person from each entity (names shown with asterisk*) shall be responsible for receiving, retaining and distributing the QAPP to their participating staff within their own organization. Contact information for the primary contact person for each participating organization is also provided in Table 1.

Table 1. Contact Information

Name	Agency, Company or Organization
<u>Aquatic Toxicology Laboratory</u>	
Primary Toxicity Testing Laboratory, Sampling Inge Werner, Linda Deanovic, Joy Khamphanh, Marie Stillway*	UCD ATL 1321 Haring Hall; University of California Davis, CA Phone: 530-754-6772 Email: mevasi@ucdavis.edu
<u>Department of Water Resources</u>	
DWR Contract Manager Kevin Reece*	DWR 901 P Street Sacramento, CA 95814
DWR Quality Assurance Officer Murage Ngatia	Phone: 916-561-0154 Email: creece@water.ca.gov Email: mngatia@water.ca.gov
<u>Haring Hall Laboratory</u>	
Collaborator: Copepod testing and histopathology Swee Teh*	VM:APC 1203 Haring Hall; University of California Davis, CA Phone: 530-754-8183 Email: sjteh@ucdavis.edu
<u>Department of Fish and Game, Water Pollution Control Laboratory</u>	
Analytical Chemistry David Crane*	DFG WPCL 2005 Nimbus Rd Rancho Cordova, 95670 Phone: 916-358-2859 Email: dcrane@ospr.dfg.ca.gov

Section A4. UCD ATL Program Organization

UCD ATL is certified by the Environmental Laboratory Accreditation Program (ELAP) to conduct toxicity tests evaluating water quality. US EPA (2000, 2002) toxicity testing methods as well as other non-EPA methods are used to characterize and identify potential contaminants in aquatic samples. The quality of data generated at UCD ATL is ensured through a variety of protocols and criteria established by US EPA and/or UCD ATL. These include, but are not limited to, extensive documentation of standard operating procedures, documentation of deviations from established protocols, as well as implementation of preventative and corrective measures to meet quality assurance objectives. The lines of communication between the participating entities are outlined in Figure 1. UCD ATL organization and responsibilities are outlined in Tables 2 and 3.

4.1 Involved parties and roles

Inge Werner (UCD ATL) will serve as the Principal Investigator (PI). The PI will 1) review and approve the QAPP, 2) provide oversight on study design and development, and 3) authorize the hiring of any personnel for this project, 4) ensure payment is received for all invoices, 5) authorize the purchase of equipment related to the project, and 6) provide the contracting entity with semiannual progress reports and a final report upon completion of this project.

Kevin Reece (DWR) will serve as the Contract Manager (CM). The CM will 1) review and approve the QAPP, 2) review, evaluate and document project reports, 3) coordinate with other monitoring efforts in the study area, and 4) verify completeness of all tasks.

Linda Deanovic (UCD ATL) will serve as the Laboratory Manger (LM) and will provide oversight for all sample processing and analysis done by UCD ATL. Specific duties for the LM are to 1) review and approve the QAPP, 2) provide pricing for all lab work to be done, 3) approve the hiring of any personnel for this project, 4) authorize and approve the purchase of all supplies related to the project, and 5) ensure that all laboratory activities are completed within the proper timelines.

Joy Khamphanh (UCD ATL) will serve as the Toxicity Testing Manager (TTM). Specific duties for the TTM are to 1) review and approve the QAPP, 2) provide oversight for all toxicity testing to be done for this project, and 3) communicate any client challenges and concerns to the PI or LM in order to resolve any issues.

Dan Markiewicz (UCD ATL) will serve as the Statistician. The Statistician's specific duties will be to 1) perform data analysis and 2) construct and manage graphs, figures and tables for the final report.

4.2 Quality Assurance Officer role

Marie Stillway is the UCD ATL Quality Assurance Officer (QAO). The QAO's role is to ensure that quality control for sample processing and data analysis procedures described in this QAPP are maintained throughout the project.

The QAO will review and assess all procedures during the life of the contract against QAPP requirements and assess whether the procedures are performed according to protocol. She will report all findings to the CM and the contracting agency's QAO, including all requests for protocol amendments. The QAO has the authority to stop all actions if there are significant deviations from required procedures or evidence of a systematic failure.

4.3 Persons responsible for QAPP update and maintenance

Revisions and updates to this QAPP will be carried out by the UCD ATL QAO, under supervision of the TTM, LM and CM. All changes will be considered draft until reviewed by the CM. Finalized revisions will be submitted for approval to the contracting agency's QAO if necessary.

4.3.1 QAPP distribution

Copies of this QAPP will be distributed to all parties involved in the project. Any future amended QAPPs will be held and distributed in the same fashion. All originals of these first and subsequent QAPPs will be held on site at UCD ATL.

Table 2. UCD ATL positions and duties

Position	Person	Responsibilities
Director	Dr. Inge Werner	Overall direction of the laboratory's research
Laboratory Manager	Linda Deanovic	Organizes, coordinates, plans and designs research projects and supervises laboratory staff.
QA Officer	Marie Stillway	Ensures that the laboratory quality assurance plan and quality assurance project plan criteria are met through routine monitoring and auditing of the systems
Field Coordinator		Sample design, sampling coordination and operations
Sample Custodian	Nathan Offer	Sample storage and disposal
Toxicity Testing Manager	Joy Khamphanh	Direct client communication with Contract Managers and clients in all projects and communicating any client challenges and concerns to the Director, Manager, Sample Custodian and/or Data Manager in order to resolve any issues.
Data Manager	Dan Markiewicz	Statistical analysis, generation of summary tables to the client upon request.
Technicians	Additional Staff	Conducts toxicity tests, TIEs and measure water quality parameters

4.3 Organizational chart

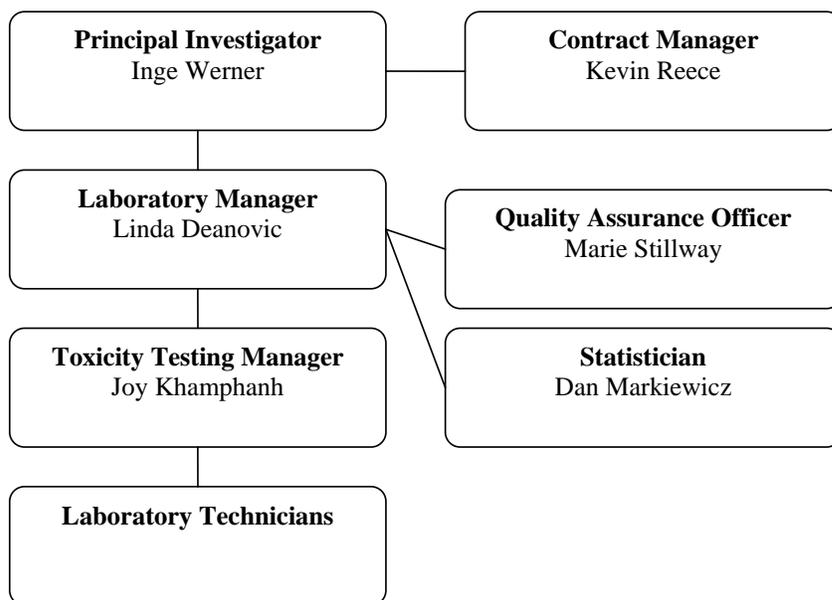


Figure 1. Organizational chart for UCD ATL

Table 3. Project Responsibilities

Responsibilities		Person
Sampling	Sampling Design	Inge Werner, Linda Deanovic; UCD ATL Kevin Reece; DWR
	Sample collection, calibration of field instruments, field analysis	UCD ATL Staff in conjunction with Department Fish and Game
	Sample delivery	UCD ATL Staff
	Sample custody/storage and lab instrument calibration	Nathan Offer; UCD ATL
Toxicity Testing	Toxicity Testing	UCD ATL Staff
	QA/QC data validation, audits and corrective actions	Marie Stillway; UCD ATL
Chemical Analyses	Safety Training	Marie Stillway; UCD ATL
	Metals, Pesticides, Others	David Crane; DFG WPCL
Chemical Analysis Quality Control and Data Validation	Metals, Pesticides, Others	David Crane; DFG WPCL
Project Direction		Inge Werner; UCD ATL Kevin Reece Ted Sommer ; DWR
Laboratory Quality Assurance		Marie Stillway; UCD ATL
Project Quality Assurance		DWR QA Officer
Contract Management		Kevin Reece; DWR
Statistical Guidance		Neil Willits, UC Davis
Data Management and Reporting		Inge Werner, Linda Deanovic, Joy Khamphanh, Dan Markiewicz; UCD ATL

Section A5. Problem Definition/Background

5.1 Problem statement

The overall goal for this study is to assess the potential for contaminated water to contribute to the observed declines of pelagic species in the Delta. The study design is building on the results of UCD ATL 2006-2007 Delta-wide monitoring project to investigate the toxicity of Delta water samples to invertebrates and early life stages of fish species of concern. In 2006-2007, water samples for invertebrate toxicity testing were collected twice a month at 15 sites characterizing primary inflows to the Delta as well as geographic regions important to pelagic fish of interest. Test results in 2007 showed acute toxicity in the lower Sacramento and Suisun Bay, and the possible presence of pyrethroids (reduced survival after synergist addition) at sites 804 (Middle of Broad Slough, west end), Suisun Bay, off Chipps Island (508) and Suisun Bay, east of middle point (504). Chronic amphipod growth effects after synergist addition were repeatedly detected in the south-eastern Delta, the lower Sacramento and Suisun Bay, indicating the presence of low concentrations of pyrethroid, or far less frequently, organophosphate (positive growth effects after synergist addition) insecticides. Several samples contained detectable concentrations of pyrethroid insecticides, primarily lambda-cyhalothrin, cyfluthrin and permethrin. The OP diazinon was detected in one sample. Delta smelt survival was reduced in two water samples from the lower Sacramento River, and molecular biomarkers quantified in juvenile striped bass exposed to Delta water samples showed sublethal stress responses in muscle, gill and liver at sites 323 and 340. Some responses were also seen at sites 910 and 609.

Comment [c1]: Should this be 14, since 323 was dropped so early on?

Comment [c2]: Once, twice, often, seldom?, some # here would be nice

Comment [c3]: What kind of responses, with striped bass r another species?

Questions to be addressed:

1. Is water in ecologically sensitive areas of the Delta toxic to delta smelt and other pelagic fish and their prey?
2. What are the causes and sources of water column toxicity in areas of the Delta for fish species of concern?
3. How sensitive are Delta species to contaminants in comparison to surrogate species commonly used in toxicity testing? Is it meaningful to use surrogate species for toxicity monitoring in the Delta?
4. Are contaminants associated with wastewater treatment effluents affecting fish species of concern?
5. Is there a relationship between toxicity results and other POD study components such as histopathologic examination of fish and *Microcystis* blooms?

5.2 Decisions or outcomes

Results from this project will provide information on acute and sublethal water toxicity in ecologically sensitive areas of the Delta. Routine partial toxicant identification tests will provide early evidence of the presence of two classes of toxic insecticides, organophosphates and pyrethroids. *In situ* tests with fish and invertebrates will provide

toxicity information under semi-realistic conditions, integrating the potential effects of site water over time. Laboratory tests with delta smelt will provide toxicity information for important sites where *in situ* testing is logistically impossible. Analysis of molecular biomarkers in larval and juvenile striped bass collected and preserved in the Delta during 2006-2007 will provide important information on the presence, as well as sublethal effects of contaminants and other stressors in the field. Comparisons of results from the species sensitivity studies will answer urgent questions regarding the relative sensitivity of Delta species of concern to contaminants of concern, facilitate toxicity monitoring results, and facilitate decision-making for future toxicity testing in the Delta. If Delta species prove to be equally or less sensitive to contaminants than standard testing species, surrogate species could be used in future studies and more tools and resources for identifying causes and sources of toxicity would become available.

5.3 Water quality or regulatory criteria

There are no water quality or regulatory criteria at this time.

Section A6. Project Description

6.1 Work statement and produced products

This project will involve the use of laboratory toxicity testing, *in-situ* toxicity testing, species sensitivity studies, and molecular field biomarkers and data interpretation to assist DWR in determining the potential for contaminated water to contribute to the observed declines of pelagic species in the Delta.

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 DWR CM with electronic semi-annual progress reports. These reports will include the number of samples processed, the number of samples analyzed, results and a timeline for the completion of the analyses. Oral progress reports to IEP project work teams will be provided as needed. An oral progress report will be provided at the IEP Annual Workshop in February 2009 and 2010. A post-field progress report that will describe the study and outcome to a peer-reviewed professional journal and/or the IEP Newsletter will be submitted. Copies of the aforementioned report will be submitted to the CM.

6.2 Constituents to be monitored and measurement techniques

Laboratory toxicity testing will involve pre-determined sub-surface grab sampling of surface waters in the Sacramento-San Joaquin Delta, coordinated through DWR and DFG and will include toxicity identification evaluations (TIEs) and chemical analyses. *In situ* toxicity tests will involve suitable locations for exposure, test protocol development and design of suitable control treatment systems. Species sensitivity studies will involve

generation of sensitivity data (96 h LC₅₀, EC₅₀, NOEC and LOEC) for comparisons between standard surrogate species used for toxicity testing and native Delta species. Molecular field biomarkers developed for striped bass in 2006-2007 will be used to detect and quantify stress responses in field-collected specimens to detect sublethal toxic effects and help identify the causative chemical(s) or other stressors.

Data obtained in this investigation will come from a combination of direct field and laboratory-derived collection methods and procedures. For the duration of the project, semi-annual progress reports, study results and interpretation will be reported in a final report. Evaluation of most data include statistical analyses.

6.3 Project schedule

Table 4. Schedule of completion dates

Activity	Completion Date
Quality Assurance Plans	April, 2008
- QA Plan for Toxicity Tests	
Toxicity Testing	December 2009
- Sample Collection	
- Reference Toxicant Testing	
- Laboratory Toxicity Testing	
- In-situ Toxicity Testing	
- Species Sensitivity Studies	
- Biomarker Analysis	
Toxicity Identification Evaluations	December 2009
- Planning	
- Dilution Series	
- Phase I TIEs	
- Analytical Chemistry	
Verification and Validation of Data	March 2010
- Final Quality Inspection of Data	
- Data Accuracy Check	
Reports	
- Semi-Annual Progress Reports to DWR	October 2008/09, April 2009
- Oral Progress Reports to IEP work teams	September 2008, 2009
- Oral Progress Report to IEP Annual Meeting	February, 2009, 2010
- Post-field Progress Report for publication	Summer, 2010
- Final Report	March 2010

6.4 Geographical setting

Sampling and *in situ* toxicity testing will be focused on sites selected from primary inflows to the Sacramento-San Joaquin Delta as well as geographic regions important to pelagic fish of interest. Sampling sites are selected based on toxicity testing results obtained in 2005-2007, and coordinated with monitoring programs of the California State Water Resources Control Board's (SWRCB) SWAMP Program, California Regional Water Quality Control Board's (RWQCB) Agricultural Waiver Program, and a SWRCB project in Suisun Marsh, "Strategy for Resolving MeHg and Low Dissolved Oxygen Events in Northern Suisun Marsh". A detailed Sampling and Analysis Plan is provided in the Appendix.

6.5 Considerations and constraints

US EPA recommends that ambient samples be collected in amber-colored glass sample containers. UCD ATL makes every effort to follow EPA recommendations; however due to the volume of water collected (in some cases up to 35 gallons/site) and method of collection (via boat), the use of glass sample containers poses significant safety risks. Therefore, for this project, amber-colored low density poly-ethylene (LDPE) containers will be used for [ambient sample collection for Hyallela tests and clear LDPE containers for chemistry samples and Delta Smelt testing](#).

US EPA recommends that toxicity tests be initiated within 36 hours of sample collection. UCD ATL makes every effort to initiate tests within 36 hours of sample collection; however due to the intense sampling schedule of this project, samples will be initiated in toxicity tests within 72 hours of sample collection. If UCD ATL is unable to initiate toxicity tests within 72 hours, the DWR CM will be consulted. Although storage at 0-6 °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 onto container surfaces during the holding period also can result in an underestimation of toxicity and yield false negatives. Sampling will be timed to minimize holding time. UCD ATL will notify the DWR CM when samples are held for more than 72 hours. Results of tests in which samples were held more than 72 hours prior to test initiation will be specifically identified in interim and final reports.

US EPA methods recommend that the control water match the hardness of the sample being tested. However, due to the number of ambient samples tested in a single toxicity test, matching the hardness of each sample is not feasible because of space and time limitations as well as costs. Instead, UCD ATL utilizes control waters of consistent hardness for the initial toxicity test. Deionized water amended to EPA moderately hard reconstituted standards (DIEPAMHR) is used as the control for *Hyallela azteca* (*H. azteca*). Delta smelt hatchery water is used as the control for delta smelt. High and low EC controls will match the EC extremes of ambient samples. For TIEs on samples that have proven to be toxic in the initial screening, the hardness/EC of the control is adjusted to that of the sample in order to determine the effect, if any, hardness/EC has on organism response.

As *H. azteca* are obtained from an outside supplier, their arrival is subject to shipping constraints and related challenges. In the event that an organism shipment arrives late or not at all, the DWR CM will be contacted. If an organism shipment arrives in which some organisms are healthy and some are not, the UCD ATL TTM will notify the DWR CM to discuss test options. These include, but are not limited to, initiating the toxicity test with a reduced number of organisms per replicate, extending the holding time, or substituting a sample(s) collected from the same site at a later date.

Section A7. Data Quality Objectives and Acceptability Criteria for Measurement Data

All tests are conducted based on protocols outlined in “Recommended Test Conditions” (US EPA, 2000, 2002) and protocols developed at UCD ATL. Deviations from protocols must be reported to the UCD ATL QAO.

Data quality objectives for this project will consist of the following:

- Sample Processing: Accuracy, Precision, Completeness
- Toxicity Testing: Precision, Completeness, Representativeness
- Data Entry: Accuracy, Completeness

7.1 Test acceptability criteria

Test acceptability for chronic *H. azteca* 10-day water column tests requires 80% or greater survival in the controls (US EPA, 2000).

Control survival limits for *Hypomesus transpacificus* (delta smelt) and *Morone saxatilis* (striped bass) in the 2006-2007 POD project were designated at 80%. However after conducting two years of developmental toxicity testing with these species, it has been determined that delta smelt and striped bass fish species are extremely sensitive at the ages utilized at UCD ATL and 80% survival was not an attainable control limit. Therefore for 2008-2009, test acceptability criteria for chronic delta smelt and striped bass 7-day ambient toxicity tests will require 50% or greater survival in the controls.

Comment [c4]: This is rather low, maybe 70 or even 60% would be stronger.

There are no current test acceptability criteria for fish (delta smelt, fathead minnow or inland silverside) or *H. azteca in situ* toxicity tests.

Test acceptability criteria for acute 96-hour species sensitivity studies require the following:

- 50% control survival for delta smelt

Comment [c5]: See above note.

- 80% control survival for *Pseudodiaptomus forbesi* (*P. forbesi*) and *Eurytemora affinis* (*E. affinis*). Test acceptability criteria for these species have not been instituted at this time; 80% control survival is a goal.
- 90% control survival for *Ceriodaphnia dubia* (*C. dubia*), *H. azteca*, *Pimephales promelas* (fathead minnow) or *Menidia beryllina* (inland silverside)

When the control performance does not meet test acceptability criteria, the DWR CM will be notified to discuss possible follow-up and all data from the test will be evaluated and noted in interim and final reports.

Table 5. Data quality objectives

Toxicity Testing Laboratory Analysis (UCD ATL)			
Parameter	Accuracy	Precision	Completeness
pH	± 0.2 pH units	± 0.5 pH units	90%
Conductivity	± 5%	± 10%	90%
Temperature	± 0.5%	± 10%	90%
Dissolved Oxygen	± 5%	± 10%	90%
Ammonia	± 0.5%	± 10%	90%
Hardness	Standard Reference Material (SRM) within 95% CI stated by provider of material	± 10%	90%
Alkalinity	SRM within 95% CI stated by provider of material	± 10%	90%
Turbidity	SRM within 95% CI stated by provider of material	± 10%	90%
Toxicity Testing	Meet all performance criteria in method relative to reference toxicant	Statistical agreement between replicates compared to the control	90%
Data Entry	100%	NA	90%

7.2 Quality Assurance

Quality assurance measures will be included in this project to ascertain the reliability of data gathered, including whether UCD ATL toxicity testing can be duplicated, and to assess whether test species are responding typically, relative to historical test results at UCD ATL. 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.

7.2.1 Positive control tests

One positive control (i.e., reference toxicant) test will be performed monthly. NaCl will be the reference toxicant used for the *H. azteca*, *C. dubia* and fathead minnow tests. The reference toxicant for delta smelt will be determined in collaboration with the DWR CM. Routine reference toxicant tests determine test species sensitivity to a toxicant, and whether the test species is reacting typically (within a specified range) to that toxicant. For fish, these tests generally include a laboratory control and a toxicant dilution series in laboratory control water. The LC₅₀ or EC₂₅ 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 within the 95% confidence interval of the running mean. If the LC₅₀ and/or EC₂₅ fall out of the 95% confidence interval, test organism sensitivity is considered atypical and results of toxicity tests conducted within those months are considered suspect. Because of the non-standard nature of our resident fish tests, conducting routine reference toxicant tests is not possible. Instead, we will conduct reference toxicant tests with delta smelt using the LC₅₀ concentration of a contaminant (e.g. copper, ammonium) and a control to assess relative sensitivity of different batches of 45-d old fish.

Comment [c6]: New for the ATL but relevant to the Delta

7.2.2 Representativeness

In terms of laboratory toxicity testing of ambient samples, representativeness refers to the degree to which data accurately represent responses of resident populations at the sites where samples are collected. Estimating risk to indigenous aquatic biota 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 use of resident Delta species *in situ* toxicity testing will provide toxicity information under semi-realistic conditions, integrating the potential effects of site water over time. Additionally, comparison of results from the species sensitivity studies will answer urgent questions regarding the relative sensitivity of Delta species of concern to contaminants of concern. If Delta species prove to be equally or less sensitive to contaminants than standard testing species, surrogate species could be used in future studies and more tools and resources for identifying causes and sources of toxicity would become available.

7.2.3 Test sensitivity

Test sensitivity refers to the ability to distinguish a statistical difference between test organism responses in laboratory control water compared to an ambient sample. Test sensitivity is frequently expressed as the percent difference between the control and ambient sample that can be detected. The level of effect that can be detected will vary, depending on control performance, variability among replicates, test species utilized and the endpoint measured. 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 (i.e., a sample is toxic but the test does not detect toxicity). Test sensitivity can be increased by increasing the number of replicates. That, in turn, increases the costs of testing. At this time, UCD ATL determines the Percent Minimum Significant Difference (PMSD) and Minimum Significant Difference (MSD) between ambient samples and the appropriate controls in chronic *H. azteca* toxicity tests.

Comment [c7]: What about for other tests/species

7.2.4 Completeness

Completeness is a measure of the data obtained compared to the amount of data expected in a project. The toxicity data acquisition phase of a project is considered complete when all sites specified in a contract have been visited the number of times designated in a contract, the number of samples designated in a contract has been collected, and the number of toxicity tests designated in the contract have been successfully completed. UCD ATL strives for a minimum of 90% completion of data.

7.2.5 Comparability

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, UCD ATL strictly documents and adheres to US EPA test protocols, UCD ATL SOPs, QA measures outlined herein, and acceptable reference toxicant test results. Therefore, the laboratory results in one project can be compared to results from previous UCD ATL projects, as well as from other laboratories that adhere to the same US EPA protocols and QA measures.

Section A8. Special Training Requirements/Safety

8.1 Specialized training and safety requirements

Laboratory technicians are trained to conduct a wide variety of activities using standard protocols (UCD ATL SOPs, 2007) to ensure samples are analyzed in a consistent manner. All new laboratory personnel attend an initial training and laboratory safety session, and thereafter attend a bi-annual general safety review. UCD ATL field staff attend a field-specific training session and have attended a certified boat-safety training session.

8.2 Training, safety and certification documentation

Staff and safety training is documented and filed on-site at UCD ATL. Documentation consists of a record of the training data, instructor and signatures of completion. The UCD ATL QAO will certify the proficiency of ATL staff. The QAO will conduct internal lab performance audits throughout the duration of the project. Certification and records are maintained and updated by the UCD ATL QAO for all laboratory staff.

8.3 Training staff

The UCD ATL TTM trains or appoints senior staff to train personnel. The UCD ATL QAO ensures that training is given according to standard laboratory methods, maintains documentation and performs performance audits to ensure that personnel have been trained properly.

Section A9. Documentation and Records

UCD ATL generates records for sample receipt and storage, analyses and reporting. All raw toxicity test and water quality data will be recorded in non-erasable ink on standardized printed data sheets. The raw data will be entered into spreadsheets and manipulated with statistical programs, then photocopied and used when performing data interpretations. All data will be submitted to the DWR CM as part of the corresponding project reports in pre-formatted Excel spreadsheets that will include data from toxicity testing, *in situ* toxicity testing, species sensitivity tests, toxicity identification evaluations and water chemistry. All spreadsheets 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.

Section B1. Sampling Process Design (Experimental Design)

1.1 Sites and sampling schedule

Fifteen sampling sites will be selected based on toxicity testing results obtained in 2005-2007 and coordinated with monitoring programs SWRCB SWAMP, RWQCB Ag Waiver and RWQCB Suisun Marsh. Sampling will intensify in some important areas (Cache Slough/lower Sacramento, Suisun Marsh and Bay) of the Delta where acute toxicity was detected in 2007. Site locations and sampling schedule are outlined in Tables 6 and 7. Sampling details are provided in the Sampling and Analysis Plan, located in the Appendix.

1.1.1 *H. azteca* toxicity testing

Fifteen sampling sites will be collected twice a month at selected sites in the Sacramento-San Joaquin Delta from January 2008 - December 2009 (details see below). More frequent sampling will occur when acute toxicity is detected. TIEs will be performed when a sample causes $\geq 50\%$ mortality within 7 days. If a sample causes $\geq 50\%$ mortality within 96 hours, follow-up samples will be collected in an attempt to identify the sources of toxicity. Proposed follow-up collection sites are outlined in Table 8 and are discussed in more detail in the Sampling and Analysis Plan, located in the Appendix.

1.1.2 Fish toxicity testing

Samples will be collected twice a month throughout between late March and June 2008, 2009 for delta smelt testing, from 4-6 stations relevant to these species in: Suisun Bay/Marsh, Cache Slough/lower Sacramento, Hood, Napa River and Rough & Ready Island. Details are provided below.

Table 6. Site locations and sampling schedule

Station	Location	Latitude	Longitude	Day: <i>H. azteca</i> only	Day: <i>H. azteca</i> & delta smelt
340C	Napa River, Historic 340 at the seawall	38-05'-51"N	122-15'-43.9"W	Weds.	Thurs.
405	Carquinez Straight, just west of Benicia arm dock	38-02'-22.9"N	122-09'-01.8"W	Weds.	Thurs.
Suisun ^{A,C}	Suisun Slough downstream of Boynton Slough	38-12'-28.2"N	122-01'-56.9"W	Tues.	Weds. (by car)
508	Suisun Bay, off Chipps Island, opposite Sac. North Ferry Slip	38-02'-43.8"N	121-55'-07.7"W	Weds.	Thurs.
602	Grizzly Bay, northeast of Suisun Slough at Dolphin	38-06'-50.4"N	122-55'-46.3"W	Weds.	Thurs.
609	Montezuma Slough at Nurse Slough	38-10'-01.9"N	121.56'-16.8"W	Weds.	Thurs.
711	Sacramento River at the tip of Grand Island	38-10'-43.7"N	121-56'-55.1"W	Thurs.	Weds.
Light 55 ^C	Sacramento River Deep Water Channel at Light 55	38-16'-26.5"N	121-39'-13.6"W	Thurs.	Weds.
Hood ^{B,C}	DWR water quality monitoring station	38-22'-03.6"N	121-31'-13.6"W	Fri.	Tues. (by car)
Cache-LinC	Confluence of Lindsey Slough/Cache Slough	38-14'-39.2"N	121-41'-19.5"W	Thurs.	Weds.
Cache-UI	Upper Cache Slough, mouth of Ulatis Creek	38-17'-02.7"N	121-43'-04.3"W	Thurs.	Weds.
815	San Joaquin, Confluence of Potato Slough	38-17'-01.5"N	121-34'-21.5"W	Thurs.	Weds.
902	Old River at mouth of Holland Cut	38-01'-09.1"N	121-34'-55.9"W	Thurs.	Weds.
915	Old River, western arm at railroad bridge	37-56'-33"N	121-33'-48.6"W	Thurs.	Weds.
R&R ^{B,C}	San Joaquin, Rough & Ready Island			Fri.	Weds. (by car)
Napa	Napa River in Napa City at end of River Park Blvd.	38-16'-39.7"N	122-16'-56.9"W	Tues.	Weds. (by car)

A: Until boat is available, sampling will occur at Rush Ranch site near the Patwin Hut on the Marsh Trail using a portable pump system. If Rush Ranch site is inaccessible for any reason, sampling will occur at the Suisun Public Dock at the end of Maple St. in Suisun City using a portable pump system.

B: In 2009, these sites will be tested using *in situ* exposures.

C: These sites will be tested using delta smelt.

Table 7. Proposed follow-up sample collection

Comment [c8]: Significant changes to this list

Station	Location	Follow-up Sampling
340	Napa River, Historic 340 at the seawall	Resample of 340
405	Carquinez Straight, just west of Benicia army dock	Resample of 405; Pacheco Creek
Suisun	Suisun Slough, downstream of Boynton Slough	Resample of Suisun; Upstream Boynton Slough, upstream Rush Ranch
508	Suisun Bay, off Chipps Island, opposite Sac. North Ferry Slip	Resample of 508; upstream Sac River, upstream San Joaquin River, 602
602	Grizzly Bay, northeast of Suisun Slough @ Dolphin	Resample of 602; Suisun, 609, 508, 405
609	Montezuma Slough at Nurse Slough	Resample of 609; Nurse Slough, Mouth at Van Sickle Island
711	Sacramento River at the tip of Grand Island	Resample of 711; 704, Sac River near Locke, Gate from Moklumne
Light 55	Sacramento River Deep Water Channel at Light 55	Resample of Light 55
Hood	DWR water quality monitoring station	Resample of Hood
Cache-Lin	Confluence of Lindsey Slough/Cache Slough	Resample of Cache-Lin; Lindsey Slough, Cache-UI
Cache-UI	Upper Cache Slough, mouth of Ulati Creek	Resample of Cache-UI; upstream Ulati Creek
815	San Joaquin, Confluence of Potato Slough	Resample of 815; Mokelumne Slough, Potato Slough, upstream San Joaquin River, San Joaquin River to Franks Tract Connector, 812
902	Old River at mouth of Holland Cut	Resample of 902; 815, 915, Connection Slough
915	Old River, western arm at Railroad Bridge	Resample of 915; North Woodward Island, 902, Rock Slough
R&R	San Joaquin, Rough & Ready Island	Resample of R&R; Calaveras, Port of Stockton, upstream San Joaquin River, French Camp
Napa	Napa River in Napa City at end of River Park Blvd.	Resample of Napa

Section B2. Sampling Methods

2.1 Collection via boat

Sites collected on Wednesdays and Thursdays (405, 340, 602, 609, 508, Light 55, Cache-Lin, Cache-U1, 711, 815, 902, and 915) will be collected from mid-channel sites by boat. The DFG will supply UCD ATL field staff with a boat, a boat operator and whenever possible a field person to collect water samples in the Delta. Water samples will be pumped from a depth of approximately 0.5 m, transported and preserved following protocols outlined in UCD ATL SOPs 5-1 and 5-2 (UCD ATL, 2007).

In the event that follow-up collection is required at sites sampled by boat, UCD ATL will coordinate sampling. Follow-up collection will occur by boat, if possible, on loan from DFG and operated by properly trained UCD ATL field staff. Follow-up samples will be collected following the boat sampling protocols described above. Sampling protocols are described in greater detail in the Sampling and Analysis Plan in the Appendix.

2.2 Collection via vehicle

Samples from sites Suisun, Napa, R&R, and Hood will be collected by car on Tuesdays and Fridays, because boat access is unavailable at these sites. Where possible, water samples will be pumped from a depth of approximately 0.5 m. Samples will be transported and preserved following sampling protocols outlined in UCD ATL SOPs 5-1 and 5-2 (UCD ATL, 2007).

2.3 Sample containers and volumes

Sample containers will be labeled with site identification code and collection date. The sampling team will record relevant information in the field log book and on the chain of custody 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, DO, and SC], (6) sampling conditions, and (7) deviations. Toxicity test water renewals will use the initial sample.

2.3.1 *H. azteca*

Water samples for *H. azteca* 10-day chronic water column toxicity tests will be collected in seven one-gallon amber-colored LDPE cubitainers for initial screening tests, and one clear one-gallon LDPE cubitainer for possible TIE follow-up. Sample containers will be labeled with the information listed in 2.3.

2.3.2 Fish

Water samples for delta smelt 7-day toxicity tests will be collected in seven [clear](#) five-gallon LDPE cubitainers for initial screening tests. Sample containers will be labeled with the information listed in 2.3.

Section B3. Sample Handling Custody

Toxicity testing samples will be collected by the UCD ATL field team, who is responsible for collecting and delivering samples to UCD ATL. Sample containers will be packed in ice chests with sufficient blue or wet ice to maintain the US EPA 0-6 °C criterion. After collection, samples will be delivered to the laboratory as soon as possible (e.g., that day) to meet all designated holding time requirements.

Upon arrival at UCD ATL, sample temperature will be measured. If sample temperature at arrival exceeds the criterion, or if ice has formed, the CM will be contacted to determine testing procedures. Water samples will be stored at UCD ATL in the dark in environmental chambers maintained between 0-6 °C. The receipt of all samples will be logged in the appropriate logbook, and the COC forms signed and filed on site at UCD ATL.

Section B4. Analytical Methods

UCD ATL uses methods based on protocols developed by US EPA (2000, 2002) and UCD ATL SOPs (UCD ATL, 2007). Chronic 10-day water column toxicity testing for *H. azteca* (an epibenthic amphipod) is based on sediment protocols outlined in US EPA's Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates (US EPA, 2000) and UCD Granite Canyon's Standard Operation Procedures Manual (Granite Canyon, 2004). Standardized assays involving delta smelt do not currently exist. Chronic 7-day toxicity testing for delta smelt is based on chronic toxicity test protocols adapted from US EPA's Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms (US EPA, 2002), and UCD ATL protocols (2007). Aspects of these procedures that differ from the US EPA methods and the rationale for using them are outlined below.

While EPA methods do not specifically recommend aeration, UCD ATL protocols include aeration. This deviation is employed because the ambient samples generally tested at the UCD ATL require aeration to prevent oxygen super-saturation. Aeration time will be limited to minimize the loss of potential toxicity due to a volatile toxicant.

Toxicity tests are conducted with and without piperonyl butoxide (PBO). Tests with 25 ppb PBO are included because of this chemical's synergistic/antagonistic action with pyrethroid/organophosphate insecticides. Tests can provide early evidence of the presence of said chemicals.

Growth is generally lower in laboratory control water than in ambient samples due to the lack of microorganisms naturally preset in Delta water. These are an important food source for *H. azteca*. To compensate for the lack of nutrients in DIEPAMHR, a 1% delta water nutrient concentrate is added to the laboratory control and its PBO addition counterpart treatment. The nutrient concentrate consists of (up to) seven ambient water samples with ECs below 1000 uS/cm, centrifuged to 100 times the original concentration. The water samples used for centrifugation are saved from previous tests after proving to be nontoxic to *H. azteca*. This nutrient concentrate is added to the test the controls at 1% (or 1 ml to 100 ml sample). An additional control treatment of DIEPAMHR without the nutrient concentrate is included to evaluate the effects of the delta water concentrate on the organisms.

If an ambient sample causes $\geq 50\%$ mortality within 96 h, follow-up samples will be collected in an attempt to identify the sources of toxicity. Follow-up samples will be initiated in secondary initial screening toxicity tests with *H. azteca*. Appropriate sites for follow-up sampling will be determined early in 2008 using land use and point source information. This type of information will be assembled to identify potential sources of contaminants. Proposed follow-up sample collection is outlined in section B1, and discussed in greater detail in the Sampling and Analysis Plan, located in the Appendix.

If an ambient sample causes $\geq 50\%$ mortality within 7 days, toxicity identification evaluations and chemical analyses will be used to identify the toxicant(s) in question.

4.1.2 *H. azteca* toxicity identification evaluations

If any significant decrease in survival or growth is detected, water samples are sent to the DFG WPCL laboratory in Rancho Cordova for chemical analysis. A toxicity identification evaluation (TIE) test is initiated if there is 50% or greater mortality of test organisms within 7 days.

The purpose of Phase I TIEs is to identify the class(es) of contaminant(s) causing the toxicity in an initial screening test. Phase I TIE procedures provide information on the physical and/or chemical characteristics of the toxin in the toxic sample. For instance, is the chemical volatile, filterable, reducible, non-polar or pH sensitive? Phase I TIEs will consist of manipulations including but not limited to low temperature (15 °C), Disodium Ethylenediamine Tetraacetate (EDTA) addition, Piperonyl Butoxide (PBO) addition, air-stripping and solid phase extraction (SPE). If the decision is made to perform a Phase I TIE, the toxic sample will be retested to confirm toxicity.

SPE columns primarily remove non-polar organic chemicals from ambient samples. A toxic sample is passed through an SPE column and the through-column “rinsate” is tested along with the unmanipulated sample. Control water is also passed through an SPE column and serves as one of the method controls (method blank). The adsorbate is then eluted with methanol and the “eluate” added to control water and tested along with the appropriate method control(s). If the toxicant is a non-polar organic chemical, the ambient sample and control water amended with methanol eluate will exhibit mortality while the ambient sample passed through the SPE column (rinsate) results in reduced or alleviated mortality.

Heavy metals can be toxic to aquatic species if concentrations exceed threshold levels. EDTA binds to various metals, making them unavailable to biota. Three concentrations of EDTA will be added to toxic samples and tested along with the appropriate controls. If the toxicant is a metal(s), the unmanipulated sample will exhibit high mortality while the sample amended with EDTA results in reduced or alleviated mortality.

Air stripping reduces or removes toxicity caused by chemicals such as surfactants, chlorine and/or ammonia from waters. Toxic samples will be air stripped and tested along with the appropriate controls. If the toxicant is a volatile, the ambient sample will exhibit high mortality while the air stripped sample results in reduced or alleviated mortality.

Toxic samples are amended with PBO to inhibit or reduce toxicity caused by metabolically activated organophosphorous (OP) insecticides such as diazinon, chlorpyrifos and malathion (Bailey *et al.*, 1996). However, if the toxicant is a pyrethroid insecticide, such as lambda-cyhalothrin or Permethrin, the addition of PBO can synergize or increase toxicity in the PBO-manipulated sample. The unmanipulated sample and the sample amended with PBO are tested along with the appropriate controls. If the toxicant is a metabolically activated OP insecticide, the unmanipulated test sample will exhibit high mortality while the test sample amended with PBO results in reduced or alleviated mortality. If the toxicant is a pyrethroid, both the manipulated and unmanipulated samples will exhibit high mortality, as will the “rinsate” that was passed through the SPE column.

Low temperature testing increases the toxicity of contaminants such as pyrethroid insecticides, and the procedure is used to obtain additional weight of evidence for the presence of pyrethroids at potentially toxic concentrations.

If mortality of test organisms is decreased by more than 20% in the manipulated water sample, then a specific class of chemicals is assumed to cause toxicity. Improved organism performance following TIE manipulation is defined as the absence or a delay of mortality by greater than or equal to 24 h.

4.2 Delta smelt (*Hypomesus transpacificus*)

Animals are obtained from the Delta Smelt Hatchery (Tracy, CA) Delta Smelt are hatched and raised in large tanks containing treated water pumped directly from the Delta. Fish are acclimated to laboratory conditions for 48 h (see below) prior to test initiation. Tests are initiated within 72 h of sample collection.

4.2.1 Control water, fish collection and transport

Control Water: Water collected from the delta smelt hatchery is used for all control and acclimation treatments. This water is pumped directly from the intake channel of the H.O Banks Pumping Facility near Byron, CA, then passed through a series of sedimentation beds containing natural vegetation to allow any suspended solids in the water to precipitate. This less turbid water is then exposed to an ozonation system to kill any potentially harmful microbes. One day before fish are collected for testing, about 340 gallons of ozonated water are transported to UCD ATL, and appropriate control waters (see below) are prepared for the tests.

Fish Collection and Transport: Fish are maintained in large flow-through tanks at the Byron Hatchery. Using a drain valve, the water level is dropped to approximately one third of the initial volume of water to increase fish density and thus facilitate collection of the fish. One liter beakers are used to scoop up fish. These are then gently poured into a 11" x 15" metal pan containing ~ ½ inch of water. When the pan contains approximately 30- 40 fish they are gently poured into black plastic buckets containing hatchery water at a depth of 3-4 inches. Once the desired fish number is reached, the transport bucket is filled to the brim with hatchery water and bucket lids are sealed to prevent water leakage. Buckets are loaded into coolers packed very lightly with ice to keep the water temperature at 14-16 °C. Small pieces of foam are placed around the buckets to reduce vibration during transport. Fish are then transported to the UCD-ATL in Davis. Ice in coolers is replenished periodically during transport to maintain a water temperature of 14-16 °C. EC and SC are measured, and dissolved oxygen content was initially monitored during transport. It was determined that it is not necessary to aerate the water during transport.

4.2.2 Testing Procedures

Upon receipt at UCD ATL, the animals are put into a temperature-regulated water bath maintained at 16 °C. One-liter beakers are used to collect fish from the buckets, and fish are gently poured into a metal pan containing ~ 1/2" of water. The fish are gently scooped up using 100 ml beakers and released into the replicate tanks at random, submerging the beaker and allowing fish to swim freely into the tanks. Numbers of fish loaded into each tank are recorded.

Tests will be set up with approx. 30-60-day old larvae. Upon arrival at UCD ATL, 12 fish are immediately placed into the test tanks with no secondary holding units, for EC acclimation. During acclimation and testing, fish are fed three times a day with 1 ml of

Artemia and 1 ml of rotifers at each feeding. Just before test initiation, the salinity adjusted control water is drawn down from 7 liters to approximately two liters to allow for an accurate count of living fish.

Comment [c9]: Just artemia correct

Hatchery water and EC-adjusted hatchery water will be used as acclimation and control water. EC is adjusted with distilled water (Low EC Control) or seawater (High EC Control) to match the ambient water samples. Water quality parameters (EC, pH, temperature, DO and ammonia concentration) are measured daily, and dead fish are counted and removed daily. The feeding behavior of fish is monitored throughout the duration of the test. At test termination, surviving fish are counted.

4.2.3 Delta smelt Exposure System

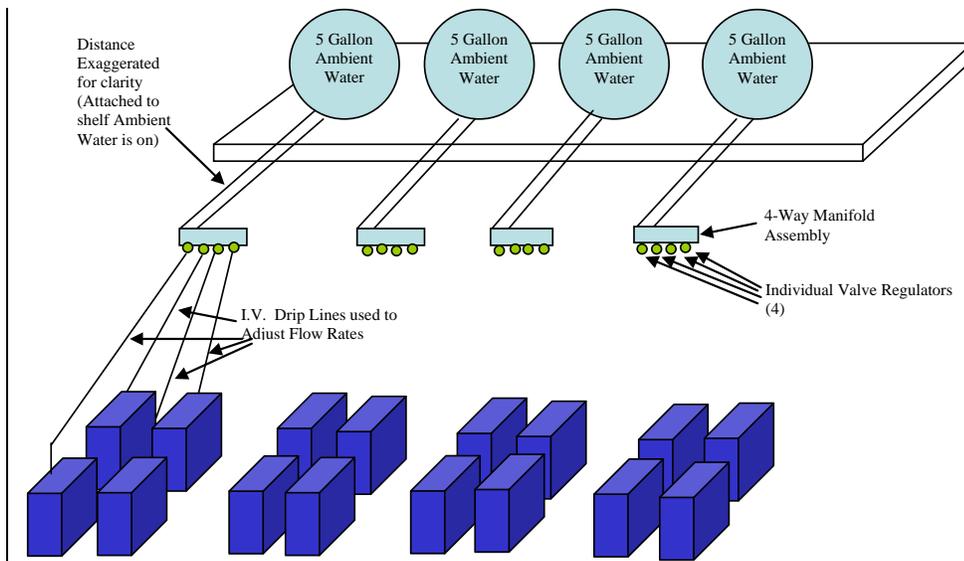


Figure 2. Tank and Manifold Assembly Schematic Diagram

4.3 *In situ*

In situ tests with fish (delta smelt, fathead minnow or inland silverside) and invertebrates (*H. azteca*) will be conducted at sites Hood and Rough & Ready Island to expose test species to water in the field and integrate potential water toxicity over time.

During 2008, the *in situ* prototype will be tested two or three times at the Hood site only so that any needed modifications can be made prior to the 2009 Smelt season. During 2009, the device will be utilized at both Hood and the Rough and Ready Island sites.

The *in situ* apparatus will consist of three main parts: the delivery system, the exposure chamber and the drainage system. A number of variables will be considered prior to the design of *in situ* devices including: availability of space at the two sites, flow to the chamber, dissolved oxygen concentrations and temperature fluctuations at the sites. The number of species tested will depend on the size of the exposure chamber, which is limited by the availability of space within the site shelters. Currently, we anticipate that we will be able to accommodate two to three species.

At each site, organisms will be placed in both an ambient exposure chamber and a control exposure chamber. The flow to the exposure chamber, dissolved oxygen concentrations and temperature in the control exposure chamber must match the same variables in the ambient chamber to the fullest extent possible. Flow rate to the chamber is important because it has the potential to physically harm the organism if the flow rate is too high. Slow flow rates could also harm the organisms by allowing metabolites to linger near the organisms longer than usual. The dissolved oxygen in the river is expected to be at or near saturation, so the control will be aerated to maintain a similar concentration. The temperature of the control exposure chamber will be maintained by a surrounding bath of ambient water, thus the temperatures should parallel one another fairly well.

The control exposure chamber will also need a few features that are not necessary for the ambient exposure chamber, primarily because it will be a recirculating system. Metabolites that will be forced through the ambient chamber with constant incoming water will have to be removed with filtration systems for the recirculating water in the control. Filtration is not avoidable due to the small volume of control water that can be housed on site and simultaneously be temperature maintained.

In situ organisms will be transported to the site in sealed containers and then gently lowered into each replicate section of the exposure chamber. Fish will be fed *Artemia* nauplii via a gravity fed drip system. *H. azteca* will be placed in their replicate cages with one or two flakes of Tetramin™ flake fish food. The ATL will count the surviving test organisms at approximately 48- and 96- h from test initiation. The food sources will be replenished at the 48 h time point as well. Vertebrate organisms will be euthanized on site after test termination.

No test acceptability criteria are in place for *in situ* testing. The ATL will not report data for EPA test species when the control survival falls below 70%. For Delta smelt, this

criterion will be reduced to 50% because the organisms are more delicate. We anticipate the handling alone may cause undetectable stresses on the organisms just prior to their deployment in the exposure chamber.

Comment [c10]: This is really low, not certain that they are that sensitive.

Temperature, pH, dissolved oxygen, and conductivity will be measured three times for each 96 h exposure, at deployment, at the 48 h survival count time point, and at test termination. In addition, an aliquot will be taken back to the lab at test initiation and termination to determine hardness, alkalinity, and total ammonia in the exposure chambers.

4.4 Species sensitivity studies

Presently the overwhelming lack of information on the toxic effects of contaminants on resident Delta species, among them delta smelt and two important prey species, *P. forbesi* and *E. affinis*, prevents an estimation of the risk of chemical contamination to pelagic organisms of concern. These sensitivity studies will provide information on the species' sensitivity to toxic chemicals relative to standard test species. Sensitivity data such as 96 h LC₅₀, EC₅₀, no observed effect level (NOEC) and lowest observed effect level (LOEC) will be generated and will be compared to the sensitivity of Delta species with that of standard toxicity test species.

4.4.1 Species

Species sensitivity studies will include the copepod (adult) species *P. forbesi*, *E. affinis*, the water flea *C. dubia*, the amphipod *H. azteca*, and fish species delta smelt, fathead minnows or inland silverside.

Copepod cultures will be maintained in the laboratory of Swee Teh. Testing will be performed according to protocols developed in Dr. Teh's lab.

C. dubia cultures will be maintained at UCD ATL, following protocols outlined in UCD ATL SOPs (2007) and recommendations in US EPA (2002).

H. azteca are obtained from an outside supplier (see 4.1). Testing will be performed according to protocols outlined in UCD ATL SOPs (2007) and recommendations in US EPA (2002).

4.4.2 Chemicals for LC₅₀ Determination

Chemicals were selected based on their known presence in the Delta, recent past, or present. Chemicals to be tested are the following: permethrin, bifenthrin, cyfluthrin, diazinon, chlorpyrifos, copper and ammonia. Test species will be exposed to five concentrations of each chemical plus appropriate controls, unless toxicity information on species sensitivity is available.

Table 9. Chemicals for species sensitivity studies

Pyrethroid insecticides	OP insecticides	Other
Permethrin	Diazinon	Ammonia (fish only)
Bifenthrin	Chlorpyrifos	Copper
Cyfluthrin		

4.4.3 Exposures

For copepods, 48 h LC₅₀s will be determined. Copepod cultures will be maintained in the laboratory, and exposure tests will be performed according to protocols developed in Dr. Teh's lab at UC Davis.

For fish, exposure concentrations to determine 96 h LC₅₀s will be determined in rangefinder tests which will consist of five concentrations and appropriate controls. There will be two replicates per treatment and five fish per replicate.

LC₅₀ exposures will consist of five concentrations and appropriate controls and/or solvent controls. There will be four replicates per treatment and 10 fish per replicate.

Acclimation to test conditions and tests will follow protocols established in 2007 at UCD ATL. For LC₅₀ tests, 45-day old delta smelt will be used, due to their higher survival rate in varying salinities and low turbidity.

Tests on standard test species (*C. dubia*, *H. azteca*, fathead minnow, and/or inland silverside) will be performed using filtered Delta water as well as laboratory control water. Tests employing delta smelt will be performed using filtered Delta water. Tests utilizing copepod species will be performed using laboratory control water.

4.5 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, EC, pH, DO are measured on all samples at test initiation and termination. DO is measured on the fresh renewal water; DO and pH are measured on the 48 h wastewater on renewal days. Ammonia-nitrogen and turbidity measurements are obtained on all ambient samples within 24 h of sample receipt; hardness and alkalinity are measured on all ambient samples within 7 days.

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; EC is measured with a YSI model [3330](#) EC meter. All meters are calibrated daily according to the manufacturers' instructions. Unionized ammonia is calculated using the formula in X. Hardness and

alkalinity are measured utilizing titrimetric methods. Instrument calibration and preventative maintenance are summarized in sections B6 and B7.

Table 10. UCD ATL analytical procedures

Analyte	Project Action Limit	Project Quantitation Limit	Analytical Method	Method Detection Limit
Alkalinity (as CaCO ₃)	NA	NA	SM ^A 2320B; SOP 6-5	NA
Ammonia	< 5 mg/L	5 mg/L	SM 4500-NH ₃ F; SOP 6-3	0 ± 0.01 mg/L as NH ₃ -N
Conductivity	<15 ppt	5 µmhos	SM 2510B; SOP 8-7	0 ± 0.5%
Dissolved Oxygen (mg/L)	< 8.9 Hyalella < 10.3 Smelt < 9.5 Bass < 8.6 standard spp.	5 mg/L	SM 4500OG; SOP 8-9	0 ± 0.01 mg/L
Hardness (as CaCO ₃)	NA	0.06 mg/L based on lowest values for calcium and magnesium	SM 2340C; SOP 6-1	NA
pH	6-9 pH units	5 pH units	SM 4500H+B; SOP 8-8	0 ± 0.01 pH units
Temperature	0-6 °C sample rec. 23 ± 2 °C Hyalella 16 ± 2 °C Smelt 20 ± 2 °C Bass 25 ± 2 °C standard spp.	NA	SM 2550B	-5 ± 0.01 °C
Turbidity	NA	NA	SM 2130B; SOP 8-13	NA

^A: Standard Methods for the Estimation of Water and Wastewater, 20th edition

4.6 Statistical analyses

Each sample will be characterized by descriptive statistics including the mean response and variation among replicates.

4.6.1 *H. azteca*

Statistical analysis of *H. azteca* 10-day chronic toxicity data involves two endpoints: 10-day survival and 10-day weight. For each toxicity test a two-part analysis will be performed using JMP 5.0.1 (SAS, 2003).

In order to maximize and standardize test sensitivity and to allow the calculation of meaningful minimum significant differences (MSDs) for all tests, we will use one-way ANOVA and Tukey's multiple comparison procedures to evaluate all comparisons among waters not treated with PBO, instead of using modified US EPA statistics for multiple concentration tests. Tukey's multiple comparison procedure has greater statistical sensitivity than most of the methods involved in the US EPA protocol, and it has the advantage of evaluating all possible pair-wise comparisons between treatments, instead of being limited to comparing each treatment to one control. The US EPA protocol requires that data are tested for normality and homogeneity of variance before being tested using ANOVA. However, Zar (1996), reports that tests for homogeneity of variance perform poorly and are not recommended for testing the underlying assumptions of ANOVA, and reports that ANOVA is reliable for multi-sample testing among means, even in cases of substantial heterogeneity of variances or considerable deviations from normality. Therefore, data will not be tested for normality or homogeneity of variance before being tested with ANOVA and Tukey's procedure. Significant reductions in survival and weight in unmanipulated samples will be evaluated relative to the control of the most appropriate conductivity. In tests containing high- or low-conductivity (EC) samples (high EC >10,000 uS/cm; low EC <100 uS/cm) and a high- or low-EC control treatment, statistics will be performed separately for the normal conductivity subset of samples and the high or low conductivity subset.

Additionally, each ambient sample and control water treatment will be compared to its PBO treated counterpart by a full factorial two-way ANOVA (two-tailed alpha = 0.05). The three terms in the ANOVA were 1) the identity of test water, 2) the presence or absence of PBO, and 3) an interaction term between ambient test water and PBO presence. When there is a significant overall effect of PBO or interaction effect, a Tukey's HSD multiple comparison procedure will be performed to identify if a significant difference exists between any control or ambient water and its PBO-treated counterpart.

MSDs will be calculated for all one-way and two-way ANOVA Tukey's tests to track the sensitivity of the survival and weigh endpoints over the course of the project.

4.6.2 Delta smelt

Data from laboratory exposures of delta smelt will be analyzed using both US EPA standard statistical protocols and one-way ANOVA with Tukey's multiple comparison procedure (US EPA, 2002). The US EPA method of data analysis shows the results of the tests according to the standardized statistical method used in aquatic toxicology monitoring and regulation throughout the United States. The Tukey's procedure

complements the US EPA protocols by allowing comparisons other than each treatment paired with one control. Compared to the US EPA procedures, the Tukey's test will provide a more conservative evaluation of significant differences between samples, since it maintains the experiment-wide alpha at 0.05.

4.7 Molecular field biomarkers

Changes in the gene transcription of stress response genes in resident fish can be powerful biomarkers for the identification of sub-lethal impacts of environmental stressors on aquatic ecosystems. Molecular biomarkers developed for striped bass in 2006-2007 (Geist *et al.*, 2007) will be used to detect and quantify stress responses in field-collected specimens from 2005-2009 to detect sub-lethal toxic effects and help identify the causative chemical(s) or other stressors. Biomarker development for delta smelt will continue with the immediate aim of selecting appropriate biomarkers for use in field and in-situ studies, as well as in laboratory studies to determine cause and effect. As soon as molecular biomarkers for delta smelt are available, archived tissue samples from laboratory and in-situ exposures will be analyzed.

Cellular stress response markers for proteotoxicity (HSP70, HSP90), phase I detoxification mechanism (CYP1A1), metal-binding (metallothionein), estrogenic effects (vitellogenin) as well as immune-function and pathogen-defense (TGF-, Mx-protein, nRAMP) were established for striped bass in 2006-2007. Quantitative real-time TaqMan® PCR was used to examine tissue-specific changes in the transcriptome of liver, spleen, white muscle, anterior kidney and gills (Geist *et al.*, 2007).

Relative quantification of stress response gene transcription is as follows: The comparative C_T method was applied to quantify gene transcription of investigative stress response genes (User Bulletin #2, Applied Biosystems). Values are reported as relative transcription or the n-fold difference relative to a calibrator cDNA (i.e., average target gene transcription of control fish). Three housekeeping genes (18S, L9, GAPDH) were tested and the one revealing smallest standard deviation and most stable transcription levels over all treatments (L9) was used to normalize the target gene signals (ΔC_T) for the differences in the amount of nucleic acid added to each reaction and the efficiency of the reverse transcriptase step. The ΔC_T for each experimental sample from the exposed fish was subtracted from the ΔC_T of the calibrator, the mean target gene signal of control fish. The linear amount of target molecules relative to the calibrator was calculated by $2^{-\Delta\Delta C_T}$. Therefore, all stress response gene transcriptions are expressed as an n-fold difference relative to the calibrator. For comparisons of basic linearized transcription values between tissues of all pooled control fish, muscle tissue revealed lowest transcription levels in all stress response genes and average transcription of each stress response gene in muscle was thus used as a calibrator for other tissues.

Work to develop biomarkers for delta smelt is ongoing. A micro-array was created to identify appropriate stress-responsive genes, which will then be sequenced. Following sequencing, Taqman probes will be designed, and quantitative real-time TaqMan® PCR will be used to measure expression of these genes in delta smelt samples.

4.9 Chemical analyses

Analyses will be performed at the Department of Fish and Game, Water Pollution Control Laboratory (DFG WPCL), in Rancho Cordova.

In the field two 1L amber glass jars will be collected per site specifically for chemical analyses. One 1L glass jar will be preserved with dichloromethane (DCM) for whole water sub-samples to be analyzed for pyrethroids. One 1L glass jar will remain unmanipulated and will be used for whole water sub-samples to be analyzed for OPs. Whole water sub-samples of a toxic sample to be analyzed for total metals will be homogenized, aliquoted and preserved in HNO₃. Sub-samples of a toxic sample to be analyzed for dissolved metals will be homogenized, aliquoted, filtered through a 0.20 µm filter and preserved in HNO₃. Results from the initial screening toxicity tests and/or Phase I TIEs will determine which type of chemical analysis will be performed.

Section B5. Quality Control

UCD ATL conducts quality control through several methodologies. While these methods of quality control are not required by US EPA, they are highly recommended to identify and/or verify organism sensitivity, contamination, matrix interference and ability to duplicate results. UCD ATL tests approximately ten percent of all samples for ensuring QA/QC requirements.

5.1 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. Through precision criteria for toxicity testing have not been identified for this project, UCD ATL assesses precision through field duplicates. A field duplicate is a second sample collected in a separate container, immediately after the initial/primary test sample. Field duplicates are tested concurrently with its primary sample and the results are evaluated to determine precision of field and laboratory staff.

The relative percent difference (RPD) between duplicates is calculated on water chemistry measurements using the following formula:

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

5.2 Accuracy

Accuracy of toxicity tests cannot be directly measured because of the lack of data to support a standard organism response against comparable test results. However, inferences can be made regarding accuracy from reference toxicant tests in order to assess the sensitivity of the organisms in a known concentration of toxicant and to determine that the organisms' response is within acceptable limits.

5.3 Contamination

Trip blank samples will be included in this project to evaluate potential incidental contamination that can occur during field sampling and sample processing. A trip blank is an analyte-free water sample that is transferred into a clean sample container that is prepared in the laboratory, brought out into the field, and treated like any other collected sample throughout the course of the trip. For this project, trip blanks will be comprised of the appropriate laboratory control water for the specific species (DIEPAMHR for *H. azteca*; Hatchery water for delta smelt). A trip blank sample is in agreement when it is statistically similar to the control.

Bottle blank samples will be included to evaluate potential incidental contamination due to the sampling container. 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 will be comprised of the appropriate control water for the specific species. A bottle blank sample is in agreement when it is statistically similar to the control.

Section B6. Instrument/Equipment Testing, Inspection and Maintenance

Laboratory equipment will be checked for operation in accordance with the manufacturer's specifications. This includes battery checks, routine replacement of membranes and cleaning of conductivity electrodes. DO membranes are replaced monthly and batteries are replaced as needed.

UCD ATL maintains its equipment in accordance with its SOPs, which include those specified by the manufacturer and those specified by the method. These SOPs have been reviewed by the UCD ATL QAO.

Section B7. Instrument/Equipment Calibration and Frequency

Laboratory instruments are calibrated, standardized and maintained according to procedures detailed in the UCD ATL SOP manual (2007). Section 8 of the manual, "Instrument Protocols", identifies step-by-step calibration and maintenance procedures. All meters undergo a daily calibration against known standards. EC and pH meters are

externally calibrated against known standards monthly for precision. Prior to use, field instruments are calibrated and recorded in the field logbook. Instruments and types of calibration required are listed below.

Mettler AE100 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 at the time the balance has been unplugged and/or moved. Prior to use the balance is checked with certified reference weights. The balance is serviced and calibrated by Quality Control Service annually.

Mettler AE163 Balance: Used for the routine weighing of *H. azteca*, fish and weigh boats. Before operation the balance is verified to be level. Adjustments are made to level properly if necessary. An internal calibration is performed at the time the balance has been unplugged and/or moved. Prior to use the balance is checked with certified reference weights. The balance is serviced and calibrated by Quality Control Service 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. Digital max/min thermometers cannot be calibrated; however temperature “tid-bits” are used whenever temperature measurements must rely on digital readings.

YSI Model 33 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 monthly with two traceable conductivity calibration standards. The probe is also checked and cleaned weekly for 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). pH meter probes are checked weekly for algal buildup and KCl storage solutions are changed monthly.

YSI DO Meter 58: Used to determine the concentration of dissolved oxygen in a water sample. The probe is zeroed daily and calibrated in saturated, deionized water at test temperature. The probe and membrane are checked every week for bubbles and wrinkles, and the membrane is replaced monthly or sooner if necessary.

HACH 2100P Turbidimeter: Used to determine the nephelometric turbidity units (NTUs) of an ambient sample. Prior to use, the meter undergoes a calibration check with NTU standards that cover the high and low ranges of the water sample.

HACH DR/890 Colorimeter: Used to determine the ammonia content of a sample. The meter determines ionized ammonia-nitrogen, from which un-ionized ammonia is calculated. A standard and a blank are run with each use to ensure the reagents are reacting properly.

Section B8. Inspection/Acceptance of Supplies and Consumables

All supplies will be examined for damage as they are received. UCD ATL ordering personnel will review all supplies as they arrive to ensure the shipment is complete and intact. All chemicals are logged in to the appropriate logbook and are dated upon receipt. All supplies are stored appropriately and are discarded upon the expiration date.

Section B9. Non-Direct Measures

The only non-direct measurements are from UCD ATL's database of data from prior studies and any needed library research. The data will be reviewed against the data quality objectives stated in section A7, and only that data meeting all of the criteria will be used in this project.

Section B10. Data Management

All data will be maintained and managed as established in section A9.

Section C1. Assessments and Response Actions

The DWR CM or his designee (e.g., DWR QAO) 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 requests a 24 h notice prior to the inspections.

If an audit discovers discrepancies or protocol deviations, the DWR QAO will discuss the observed discrepancy with the person(s) responsible for the activity (see organizational chart). The appropriate parties will discuss the accuracy of the information collected, the cause(s) of deviation(s), possible impact(s) on data quality and possible corrective actions.

1.1 Deviations and corrective actions

Tests are conducted according to procedures and conditions recommended by US EPA (2000, 2002), with the exception of those reported herein. Beyond those identified, deviations from these recommended conditions are reported to the UCD ATL QAO. The PI and DWR CM will be notified of these deviations.

In the event of a SOP/QAPP deviation or corrective action, a deviation/corrective action form will be prepared, completed, signed and the DWR CM notified. Best professional

judgment will be used in interpretation of results obtained when protocol deviations have occurred. All deviations and associated interpretations will be reported in interim and final reports. Protocol amendments will be submitted to the DWR CM and QAO. Upon approval, protocol amendments will be employed.

Section C2. Reports to Management

The following products are to be delivered by UCD ATL:

1. Semi-annual electronic progress reports to the DWR CM. The reports are to include the number of samples processed, the number of samples analyzed, results and a timeline for the completion of the analyses.
2. Oral progress reports to IEP project work teams by September 2008 and September 2009
3. Oral progress report at the IEP Annual Meeting in February 2008 and February 2009
4. A post-field progress report that will describe the study and outcome to a peer-reviewed professional journal and/or published in the summer 2009 IEP Newsletter; and submit two (2) copies of results on said research to the DWR CM.

Section D1. Data Review, Verification and Validation

Data generated by project activities will be reviewed against the data quality objectives cited in section A7, and the QA/QC practices cited in sections B5, B6 and B7. Data will be separated into three categories:

1. Data meeting all data quality objectives
2. Data meeting data quality objectives, but failing to meet precision criteria
3. Data failing to meet accuracy criteria

Data meeting all data quality objectives, but failing to meet QA/QC criteria will be flagged until the impact of the failure on data quality is determined. Once determined, the data will be moved into either the first category or the third category. Data falling in the first category is considered usable by the project. Data falling in the third category is considered not usable. Data falling in the second category will have all aspects assessed. If sufficient evidence is found supporting data quality for use in this project, the data will be moved to the first category, but appropriately flagged.

Section D2. Verification and Validation Methods

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 QAO or designee. Additionally, the QAO, or designee, 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 calibrations have been met. At the discretion of the PI, data that do not meet these requirements will either not be reported or will be reported with an explanation of any necessary conditions.

Section D3. Reconciliation with User Requirements

While this project does not have a completeness requirement at this time, UCD ATL strives to adhere to a minimum of 90% completeness. The total number of data points generated by this project may or may not be adequate to perform trend analyses and other procedures to determine the impact on the water body in question. An insufficient number of data points obtained could result in an inability to provide these assessments.

List of Acronyms and Abbreviations

CM	Contract Manager
COC	Chain of Custody
DFG WPCL	Department of Fish and Game; Water Pollution Control Laboratory
DO	Dissolved Oxygen
DQO	Data Quality Objectives
DWR	Department of Water Resources
EC	Electrical Conductivity
EC ₅₀	Concentration where 50% of organisms show an effect
EDTA	Ethylenediamine Tetraacetate
HNO ₃	Nitric acid
IEP	Interagency Ecological Program
LC ₅₀	Lowest concentration where 50% of organisms exhibit mortality
LDPE	Low Density Polyethylene
LM	Laboratory Manager
LOEC	Lowest concentration where organisms show an effect
MSD	Minimum Significant Difference
NaCl	Sodium chloride
NOEC	No observed effect concentration
OP	Organophosphorous
PBO	Piperonyl Butoxide
PI	Principal Investigator
PMSD	Percent Minimum Significant Difference
POD	Pelagic Organism Decline
PVC	Polyvinyl chloride
QA/QC	Quality Assurance/Quality Control
QAO	Quality Assurance Officer
QAPP	Quality Assurance Project Plan
RWQCB	Regional Water Quality Control Board
SOP	Standard Operating Procedures
SPE	Solid Phase Extraction
SWAMP	Surface Water Ambient Monitoring Program
SWRCB	State Water Resources Control Board
UCD ATL	University of California, Davis Aquatic Toxicology Laboratory
US EPA	United States Environmental Protection Agency
VM:APC	Veterinary Medicine: Anatomy, Physiology, Cell Biology

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Appendix