**Evaluation of Gene Expression for Sediment Toxicity Identification Evaluation**
**Work Plan**
**March 5, 2010**

Principal Investigators:
Steven Bay, Southern California Coastal Water Research Project, Costa Mesa, CA
Chris Vulpe, University of California Berkeley, Berkeley, CA

## OVERVIEW

Sediment toxicity is present throughout San Francisco Estuary, but efforts to identify the cause of toxicity using the Toxicity Identification Evaluation (TIE) process have had limited success. The limitations are related to multiple factors, including the difficulty of isolating the cause of toxicity when complex mixtures of organic contaminants are present. New methods are needed to address these limitations so that the priority chemical stressors in the Estuary can be identified. Recent advances in biotechnology have resulted in the development of rapid and cost effective methods to simultaneously measure the activity of thousands of genes in an organism, including species commonly used for toxicity testing. Research on various aquatic species has shown that changes in patterns of gene expression are sensitive and specific indicators of chemical exposure and effects. This project will utilize these methods and build upon complementary research efforts by RMP and SCCWRP to develop and test a new sediment TIE method based upon gene expression.

The objective of this project is to evaluate the effectiveness of a gene expression analysis as a method for identifying the cause of sediment toxicity in San Francisco Estuary. A microarray for measuring the expression of several thousand genes in the estuarine amphipod *Eohaustorius estuarius*, which is widely used for sediment toxicity testing, has been developed in the laboratory of Dr. Vulpe at UC Berkeley. Three tasks will conducted in this project to develop and test a TIE method based on the microarray:

1) Calibration of molecular TIE for sediment contaminants
2) Gene expression analysis of evaluation samples
3) Evaluation of molecular TIE

Completion of the project is expected by December 31, 2010. The products of the research are expected to include a publication describing new methods for identifying the cause of sediment toxicity to E. estuarius and the results of applying the method to contaminants and sediment samples of relevance to San Francisco Estuary.

## BACKGROUND

Sediments in marine ecosystems accumulate numerous contaminants from point sources (e.g., municipal and industrial effluents) and non point sources (e.g., urban and agricultural runoff) and may become toxic to sediment dwelling organisms. The assessment of sediment quality is required by many monitoring and regulatory programs. Sediment toxicity tests with sediment dwelling organisms, such as the marine amphipod *Eohaustorius estuarius*, are frequently used to

assess sediment quality along the west coast of North America. However, the use of sediment toxicity information to assist in the development of management actions (e.g., source control, sediment remediation) is limited by the difficulty of determining which contaminants are responsible for the biological effects. This difficulty is due to the presence of complex mixtures of contaminants at most sites and limitations in the ability of chemical measurements to reliably determine the bioavailable fraction of toxicants. The Toxicity Identification Evaluation (TIE) process is used to determine the causal agents in environmental samples. This process uses a variety of chemical/physical separation methods and treatments to remove one or more toxicant classes, coupled with toxicity testing following each manipulation. Sediment TIE methods have been most successful in differentiating broad classes of toxicants in sediments where a high level of acute toxicity is present and when the toxicity is caused by a single type of contaminant, such as pyrethroid pesticides.

Sediment toxicity to the amphipod *Eohaustorius estuarius* is present throughout San Francisco Estuary, but recent sediment TIE investigations have had limited success in identifying the responsible contaminants. The limitations to the effectiveness of existing sediment TIE methods are related to a variety of factors, including: 1) most toxicity is at a low to moderate level where the methods are unreliable; 2) complex mixtures of organic contaminants are present that are difficult to separate from one another for evaluation; and 3) lack of chemical-specific effects thresholds for sediment. Ongoing research sponsored by RMP and the Southern California Coastal Water Research Project (SCCWRP) is helping to address some of these limitations. The RMP research includes conducting a series of laboratory experiments with selected contaminants that will be used to refine TIE methods for organics and develop effect thresholds for sediment associated contaminants; this research will improve the effectiveness of traditional sediment TIE methods. SCCWRP's TIE research includes a project to develop a new type of TIE method that is based on measuring changes in gene expression of the test organism, known as a molecular TIE[1,2]. The SCCWRP molecular TIE research is being conducted in collaboration with Dr. Chris Vulpe (UC Berkeley) and uses changes in gene expression as assayed by an expressed genome microarray in the marine amphipod *Eohaustarius estuarius*, the same species used in the RMP TIE research. Recent research has demonstrated that aquatic organisms produce distinctive patterns of gene expression in response to contaminant stress, and that these patterns can be used in a diagnostic manner to investigate the cause of toxicity [3,4]. The molecular TIE approach has the potential to greatly enhance the success and applicability of sediment TIEs, yet the method must first be developed and evaluated. SCCWRP's research program will develop a gene microarray for *E. estuarius*, which is the first phase of molecular TIE development, but resources are not available to adapt and evaluate this tool for use in TIEs.

The RMP and SCCWRP TIE research programs are complementary and have the potential to benefit multiple agencies. These ongoing projects also provide an opportunity to accelerate molecular TIE method development in a cost effective manner. The products from this study will benefit multiple agencies responsible to protecting and restoring water quality in marine environments..

**Work Plan**

This project will be conducted as a collaboration among SCCWRP, UC Berkeley, and RMP. Samples from concurrent RMP and SCCWRP monitoring and TIE research will be used to help develop and a molecular TIE for *E. estuarius* based on a gene microarray. This project will consist of three tasks:

**Task 1: Calibration of molecular TIE for sediment contaminants**

To identify and monitor specific chemicals, a robust data interpretation framework is needed that uses the gene expression information to determine the type and magnitude of contaminant exposure. We will first determine gene expression profiles for *E. estuarius* exposed to individual contaminants in water under controlled laboratory conditions. We will then identify a set of differentially expressed genes which respond in a specific, robust and dose responsive manner to each contaminant in each organism.

This task will utilize samples of *E. estuarius* obtained from laboratory exposure studies conducted by SCCWRP and MPSL for contaminants of high concern for sediment toxicity identification. Gene expression profiles will be developed for single chemicals representing three classes of contaminants: pyrethroid pesticides (e.g., cyfluthrin), chlordane, polycyclic aromatic hydrocarbons (e.g., pyrene). Samples from a variety of doses, ranging from no effect to 50% mortality, will be analyzed. The RNA of replicate samples of animals from each exposure treatment will be extracted and applied to a gene microarray developed by SCCWRP/UCB that is expected to contain probes for approximately 15 thousand amphipod gene transcripts. The microarrays will be washed and scanned with a laser-based detection system (e.g. Agilent, Palo Alto, CA). We will compare gene expression in toxicant exposed and control organisms utilizing standard protocols (Agilent, Palo Alto, CA). For each gene, the relative expression in the exposed as compared to control will be determined.

Methods developed in the Vulpe laboratory will be used to identify differentially expressed genes that are specific to each contaminant[5]. Based on previous experience, we estimate that 100-500 genes will be differentially expressed for each contaminant. We will apply a variety of computational analysis tools to identify a set of differentially expressed genes which uniquely identify exposure to a particular contaminant. We analyze the differentially expressed genes for each contaminant to identify transcripts which are dose responsive. **Deliverables:** The end-product will be a specific gene expression profile for each contaminant.

**Task 2: Gene expression analysis of evaluation samples**

Analyses conducted in this task will help determine whether the contaminant-specific gene markers identified in Task 1 are reliable and sensitive indicators of sediment toxicant exposure. This task will be accomplished by analyzing RNA from blind laboratory test samples, where the contaminant type and dose level are not known by the analyst. Comparison of the analyst's determination of the toxicant type (based on gene expression pattern matching) to the actual contaminant used in the sample will be used in Task 3 to determine the accuracy of a microarray-based molecular TIE approach.

Two types of samples will be analyzed in this task: laboratory-spiked samples and field samples from monitoring programs. The spiked samples of will be obtained from water and sediment experiments conducted at MPSL and SCCWRP to determine the effect thresholds of specific chemicals. *E. estuarius* will be exposed for 10 days (standard sediment test duration) to either water or sediment spiked with known concentrations of singe chemicals. Replicate samples of amphipods from treatments representing the control and varying levels of toxic response (e.g., LC5, LC20, LC50) will be preserved for gene expression analysis. RNA will be extracted, purified, and applied to the microarray using similar methods as those for Task 1. Additional samples of amphipods exposed to other stressors, such as ammonia or low salinity, will also be tested to check for the influence of confounding factors.

Selected samples of amphipods from the 2009 RMP monitoring program will also be analyzed. These samples will represent a range of responses and locations in the Estuary, ranging from nontoxic to moderate toxicity.

A minimum of three replicates of each sample type will be analyzed using the *E. estuarius* microarray to determine the relative expression of multiple genes. We will compare the expression profiles from the samples with the profiles generated for known chemicals (Task 1). We will determine if the expression profile of the unknown samples from the spiked samples correspond to specific contaminant types and response levels.

**Deliverable:** The end-product will be an analysis of the degree of match with the known contaminant type for each set of test samples.

**Task 3: Evaluation of molecular TIE**

The results from Task 2 will be evaluated to assess the potential utility of using gene expression as a molecular TIE approach for contaminated sediments. Statistical analyses and comparisons will be conducted to examine three characteristics of the method: 1) variability, 2) accuracy, and 3) robustness.

- Variability will be measured by statistical analysis (e.g., coefficient of variation of gene fold induction) of the results for individual replicates. These results will indicate the amount of replication needed to obtain a representative measure of gene expression.
- Accuracy will be quantified as the percent agreement of the microarray-based contaminant identification with the actual toxicant present in blind samples. This analysis will be based on results for samples containing various levels of the contaminants used to calibrate the microarray in Task 1.
- Robustness of the microarray approach will be examined by comparing the results of contaminant identifications for samples that contain various types of potentially confounding factors. A robust approach will be indicated by relatively little change in accuracy when the amphipod test sample includes exposure to novel stressors not used for calibration as compared to samples free of such stressors.

A report and scientific journal manuscript will be prepared that summarizes the results of these comparisons and discusses the potential of the molecular approach for use in sediment TIEs. A description of the microarray composition and gene expression analysis methods will also be

included in the report. The draft report and manuscript will be submitted to the TRC for review and revised to address comments. It is anticipated that the final report will become a SFEI publication and the manuscript will be submitted to a leading environmental journal such as Environmental Science and Technology.

**REFERENCES:**

1. Poynton HC, Wintz H, Vulpe CD. Progress in ecotoxicogenomics for environmental monitoring, mode of action, and toxicant identification. In: Christer H, Peter K, eds. Advances in Experimental Biology: Elsevier; 2008:21-73, 322-3.

2. Poynton H, Vulpe C. Ecotoxicogenomics: Emerging Technologies for Emerging Contaminants. Journal of the American Water Resources Association 2008;45:83-96.

3. Poynton HC, Zuzow R, Loguinov AV, Perkins EJ, Vulpe CD. Gene Expression Profiling in Daphnia magna, Part II: Validation of a Copper Specific Gene Expression Signature with Effluent from Two Copper Mines in California. Environ Sci Technol 2008;42:6257-63.

4. Poynton HC, Loguinov AV, Varshavsky JR, Chan S, Perkins EJ, Vulpe CD. Gene Expression Profiling in Daphnia magna Part I: Concentration-Dependent Profiles Provide Support for the No Observed Transcriptional Effect Level. Environ Sci Technol 2008;42:6250-6

5. Loguinov AV, Mian IS, Vulpe CD. Exploratory differential gene expression analysis in microarray experiments with no or limited replication. Genome Biol 2004;5:R18.

**SCHEDULE**

**DELIVERABLES AND TIME LINE**

| **Deliverable** | **Due Date** |
|---|---|
| Task 1. Molecular TIE calibration | 6/30/10 |
| Task 2. Gene Expression analysis of evaluation samples | 10/30/10 |
| Task 3. Evaluation of molecular TIE results | 11/30/10 |
| Draft report, EEWG review, final report | 12/30/10 |

Project Duration: January 1, 2010 to Dec 30, 2010