

**Southern California Bight 1998 Regional Monitoring Program:  
IV. Sediment Toxicity**

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## FOREWORD

The Southern California Bight (SCB) is an important and unique ecological resource. The diverse habitats present in the SCB allow for the coexistence of a broad spectrum of species, including more than 500 species of fish and 1,500 species of invertebrates. The SCB is also one of the most densely populated coastal regions in the country, which creates stress upon the marine environment through activities such as contaminant discharge from effluents and nonpoint sources, fishing, and habitat modification. Over \$10 million is spent annually to monitor coastal environmental quality in the SCB. These monitoring programs provide important site-specific information about the impacts of individual waste discharges, but do not describe the condition of the SCB as a whole. Regional information is needed by resource managers to assess cumulative impacts of contaminant inputs and to evaluate relative risk among different types of stresses.

The 1998 Southern California Bight Regional Monitoring Project (Bight'98) is part of an effort to provide an integrated assessment of the SCB through cooperative regional-scale monitoring. Bight'98 is an expansion of the 1994 Southern California Bight Pilot Project (SCBPP) regional survey (SCBPP Steering Committee 1998) and represents the joint efforts of 62 organizations (Appendix A). Bight'98 is organized into three technical components: (1) Coastal Ecology, (2) Shoreline Microbiology, and (3) Water Quality. This report presents the results of the sediment toxicity portion of Bight'98, which is a part of the coastal ecology component. Copies of this and other Bight'98 reports are available for download at [www.sccwrp.org](http://www.sccwrp.org).

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## EXECUTIVE SUMMARY

Sediment toxicity measurements were conducted during the 1998 Southern California Bight Regional Monitoring Project (Bight'98) in order to accomplish three goals: (1) to determine the percent of area in the SCB that contains sediments toxic to marine organisms; (2) to compare the responses among sediment toxicity test methods; and (3) to evaluate the relationship between sediment toxicity and chemical contamination or changes in benthic communities.

Sediment from 303 sites on the continental shelf between Point Conception, California, and the United States-Mexico international border were sampled between July 13 and September 16, 1998. Sites were selected using a stratified random design. Five of the strata were located offshore (river mouths, large publicly owned treatment works [POTW] discharge areas, small POTW discharge areas, remaining shallow areas [5-30 m], and remaining mid-depth areas [30-120 m]). Three additional strata were located within bays and harbors, which included marinas, ports/industrial areas, and other harbor areas (less-developed areas that did not serve port/industrial or marina functions).

Subsets of the sediment samples were evaluated for toxicity using up to four methods. Bulk sediment from 241 stations was measured for toxicity using a 10-d amphipod (*Eohaustorius estuarius*) survival test. Sediment extracts from 268 stations were evaluated for toxicity using the P450 human reporter gene system (HRGS) test, which measures the concentration of organic compounds that induce the cytochrome P450 enzyme system (e.g., PAHs, dioxins, furans, and some PCBs). Elutriates from 173 samples were tested for sublethal toxicity (bioluminescence inhibition) to phytoplankton (*Gonyaulax polyedra*) using the QwikSed test. Interstitial water from 88 samples was analyzed for sublethal toxicity (bioluminescence inhibition) to the marine bacterium, *Vibrio fischeri* (Microtox test).

Seven laboratories conducted the amphipod survival tests. An interlaboratory comparison exercise completed prior to analysis of the Bight'98 sediment samples demonstrated that each laboratory was capable of meeting test performance objectives and providing similar toxicity results. The remaining three tests were each conducted by a single laboratory.

The amphipod test detected toxicity in each of the seven strata. Amphipod toxicity was most prevalent in bay and harbor areas, where 13-37% of the area (depending upon the stratum) was toxic. Toxicity was least prevalent in POTW outfall areas (6% of the area) and the shallow portion of the coastal shelf (3% of the area).

Each of the other tests also detected sediment toxicity in selected strata. The QwikSed test was the most sensitive of the toxicity indicators. Toxicity using QwikSed was detected in elutriate samples from bays and harbors and also from POTW outfall areas. HRGS gene activity was induced by sediment extracts from 30 stations, with most of the induction produced by samples from port/industrial or marina areas. Microtox measurements of interstitial water were

taken but were unsuitable for use because of changes in toxicity related to prolonged sample storage. The Microtox test data were not used for the assessment of sediment quality. The data for the remaining three indicators were analyzed to evaluate the relative sensitivity of each test and to provide an integrated measure of sediment quality.

While 69-78% agreement was observed between pairs of toxicity tests in classifying stations as toxic/nontoxic, different patterns of response were indicated by each test. The QwikSed test results were not correlated with either amphipod survival or HRGS response and the correlation between HRGS and amphipod survival was significant but low ( $r = 0.285$ ).

The three test responses were combined into an integrated assessment of sediment quality using a weight of evidence approach that incorporated the relative ecological relevance and severity of the test responses. The integrated assessment identified 19% (644 km<sup>2</sup>) of the SCB as areas of potential or high concern. Areas of high concern (2.7%) were almost exclusively located within harbors and bays, while areas of potential concern were present in all strata tested.

The Bight'98 amphipod toxicity results for bay and harbor strata (13-37% of the area affected) fell within the general range reported in previous local studies by the National Oceanic and Atmospheric Administration (NOAA) and the State Water Resources Control Board (14-66% of the area affected). The persistent occurrence of toxicity in port and marina areas indicates that sediment quality in many of these areas is not improving. These locations are good candidates for additional research designed to identify the cause of toxicity.

Temporal differences in toxicity were apparent in two areas of the SCB. Amphipod toxicity was less prevalent in San Diego Bay compared to samples analyzed in 1992-94 and amphipod toxicity was greater in mid-depth areas compared to samples analyzed in 1994. The cause of these temporal differences may be related to several factors, including the use of different amphipod test species and variations in sediment contaminant concentrations. Analysis of sediment chemistry data (not yet available) is needed to help determine the cause of these trends.

Sediment toxicity is just one of three types of information needed to assess coastal sediment quality. Measures of sediment contamination and biological response (e.g., benthic community impacts) are also needed to establish whether the toxicity patterns are ecologically significant and associated with anthropogenic inputs.

Additional data are also needed to evaluate the significance of the different response patterns between the amphipod, QwikSed, and HRGS tests. The variable responses among test methods may reflect differences in contaminant sensitivity between species, which was the intent of using multiple toxicity indicators. Some of the variation may also be related to differences in exposure or contaminant bioavailability caused by different laboratory test procedures. Comparisons of the toxicity results with sediment contamination and benthic community characteristics are needed to help determine the predictive ability of the test methods and verify the efficacy of the weight of evidence strategy used to integrate the toxicity results.

## Definition of Terms:

**Benzo[a]pyrene equivalents (B[a]PEq):** The results of the P450 HRGS assay expressed as the concentration of benzo[a]pyrene required to produce an equivalent toxic response.

**Bioassay:** See toxicity test.

**Bioavailability:** The fraction of a chemical present in the sediment that is available for uptake by organisms.

**Control chart:** A plot of the LC50 or EC50 values from the previous reference toxicant exposures performed by a laboratory. New tests falling between control lines representing plus and minus two times the standard deviation are considered to be within acceptable limits.

**Cytochrome P450:** Group of enzymes that transforms the structure of organic chemicals within the cells of an organism. The presence of organic chemicals may induce organisms to produce these enzymes.

**Dose-response effect:** Observed effect of different concentrations of a toxicant on bioassay test organisms. Generally expected that effect increases with concentration.

**EC50:** Concentration of a toxicant predicted to cause a sublethal effect in 50% of test organisms over the course of an exposure period.

**Elutriate:** An aqueous sample produced by mixing water with sediment, then separating the water and sediment phases. The water phase is subsequently used for testing.

**Fold induction:** The amount of cytochrome P450 induction in the human reporter gene system (HRGS) test, expressed relative to the control. It is calculated by dividing the luminescence of the sample by the luminescence of the solvent control.

**Interstitial water:** Water that is between the grains of whole sediment.

**LC50:** Concentration of a toxicant predicted to cause a lethal effect in 50% of test organisms over the course of an exposure period.

**Microtox™:** A bioassay system that utilizes luminescent bacteria to measure the toxicity of aqueous samples. Reduction in the ability of the bacteria to produce light indicates toxicity.

**Negative control:** A sample from a site known to be uncontaminated that is tested along with samples of unknown toxicity.

**POTW:** Publicly owned treatment works (wastewater treatment facility).

**P450 HRGS test:** A bioassay method in which genetically modified human cells are exposed to extracts of a sample. The presence of certain organic chemicals induces the cytochrome P450 system of the cells, which results in a bioluminescent reaction. Increased production of light indicates a higher concentration of the organic chemicals.

**QwikSed™:** A bioassay system that utilizes luminescent algae to measure the toxicity of sediment elutriates. Reduction in the ability of the algae to produce light indicates toxicity.

**Reference toxicant:** A single compound that is tested along with an unknown sample in order to determine the sensitivity of the test organisms. Comparing reference toxicant results between experiments allows for determination of the validity of each test (see control chart).

**Stratum:** A subset of stations from the stratified random sampling design. Stations within a given stratum have some characteristic in common (e.g., location near river mouths).

**Toxicity test:** A laboratory experiment that measures the response (e.g., survival, growth, or reproduction) of an organism following exposure to a sample suspected of containing harmful substances.



# TABLE OF CONTENTS

## SECTION

Toxicology Committee Members.....	ii
Foreword .....	iii
Acknowledgements .....	iv
Executive Summary.....	v
Definition of Terms .....	vii
List of Figures.....	xi
List of Tables .....	xii
 <b>I. INTRODUCTION .....</b>	 <b>1</b>
 <b>II. METHODS .....</b>	 <b>2</b>
A. Sampling Design .....	2
B. Field Methods .....	4
C. Laboratory Methods.....	4
D. Data Analysis.....	7
 <b>III. QUALITY ASSURANCE EVALUATION .....</b>	 <b>14</b>
A. Sampling Success.....	14
B. Sample Storage .....	14
C. Test Performance .....	16
 <b>IV. DESCRIPTIVE RESULTS .....</b>	 <b>25</b>
A. Amphipod Survival .....	25
B. QwikSed .....	28
C. P450 HRGS .....	31
 <b>V. COMPARISON OF INDICATORS .....</b>	 <b>34</b>
 <b>VI. REGIONAL ASSESSMENT OF TOXICITY .....</b>	 <b>37</b>
 <b>VII. DISCUSSION .....</b>	 <b>41</b>
 <b>VIII. CONCLUSIONS.....</b>	 <b>44</b>
 <b>IX. RECOMMENDATIONS.....</b>	 <b>46</b>
 <b>X. LITERATURE CITED .....</b>	 <b>48</b>

- APPENDIX A.** Participants in the Southern California Bight 1998 Regional Monitoring Program (Bight'98).
- APPENDIX B.** Interlaboratory comparison of sediment toxicity tests with the Amphipod *Eohaustorius Estuarius*
- APPENDIX C.** Microtox storage experiment
- APPENDIX D.** Test results by station
- APPENDIX E.** Toxicity results mapped by indicator
- APPENDIX F.** Relationship between sediment characteristics and indicator responses

## LIST OF FIGURES

FIGURE II-1. Locations of all stations sampled for sediment toxicity during the Bight'98 project.....	11
FIGURE III-1. Summary of cadmium reference toxicant test results for amphipod ..... survival tests.	18
FIGURE III-2. QwikSed reference toxicant results.....	20
FIGURE III-3. Concentration of ammonia in elutriate samples stored prior to QwikSed analysis. ....	21
FIGURE III-4. Dose-response plot of ammonia effects on <i>G. polyedra</i> with regression curve. ....	21
FIGURE III-5. Influence of ammonia correction procedure on QwikSed luminescence value. ....	22
FIGURE III-6. Reclassification of stations resulting from correction of QwikSed results for ammonia toxicity.....	22
FIGURE III-7. Response of the P450 HRGS assay to the reference inducer (TCDD) over the testing period for Southern California Bight'98 samples. ....	24
FIGURE IV-1. Percent of sediment samples toxic to amphipods from seven strata in the Southern California Bight. ....	26
FIGURE IV-2. Percent of sediment samples toxic to QwikSed from seven strata in the Southern California Bight. ....	29
FIGURE IV-3. Percent of sediment samples toxic to HRGS from seven strata in the Southern California Bight.....	32
FIGURE V-1. Results of toxicity classifications for each pair of toxicity tests.....	35
FIGURE V-2. Comparison of indicator responses for sediment samples. ....	36
FIGURE VI-1. Percent of Southern California Bight not toxic to each indicator.....	37
FIGURE VI-2. Percent of area (+ 95% confidence interval) found to be toxic in each stratum. ....	38
FIGURE VI-3. Percent of area of concern based upon joint toxicity classification.....	40

## LIST OF TABLES

TABLE II-1. Number of stations selected for toxicity evaluation for each stratum. ....	12
TABLE II-2. List of participating laboratories and toxicity indicators measured.....	13
TABLE III-1. Bight'98 toxicity sample collection success. ....	14
TABLE III-2. Bight'98 toxicity sample holding time. ....	16
TABLE III-3. The HRGS assay response to Santa Monica Bay reference sediment. ....	24
TABLE IV-1. Sediment samples toxic to amphipods from seven strata in the Southern California Bight.....	26
TABLE IV-2. Sediment samples toxic to amphipods from nine harbors and bays in the Southern California Bight. ....	27
TABLE IV-3. Sediment samples toxic to QwikSed from seven strata in the Southern California Bight.....	29
TABLE IV-4. Sediment samples toxic to QwikSed from nine harbors and bays in the Southern California Bight.....	30
TABLE IV-5. Sediment samples toxic to HRGS from seven strata in the Southern California Bight.....	32
TABLE IV-6. Sediment samples toxic to HRGS from nine harbors and bays in the Southern California Bight.....	33
TABLE VI-1. Percent of area in each stratum classified as toxic by indicator type. ....	38
TABLE VI-2. Strategy for determining levels of concern from results of three types of sediment toxicity tests. ....	40

## I. INTRODUCTION

The 1998 Southern California Bight Regional Monitoring Project (Bight'98) is organized into three technical components: (1) coastal ecology, (2) shoreline microbiology, and (3) water quality. The goal of the coastal ecology component of Bight'98 is to assess the condition of the bottom environment and the health of biological resources in the SCB. Assessing the health of the bottom environment requires the use of multiple indicators, usually sediment contamination, sediment toxicity, and benthic community structure. Sediment toxicity tests complement these other indicators of sediment quality by providing a measure of the joint effect of contaminant mixtures. Toxicity tests use biological responses to describe sediment quality, thus providing a mechanism to evaluate the significance of chemical contamination on marine life.

The sediment toxicity portion of Bight'98 was designed to accomplish the following goals:

- To determine the percent of area in the SCB that contains sediments toxic to marine organisms.

This study used four methods to measure sediment toxicity. Sediments were assessed using the standardized amphipod survival test, two test methods measuring physiological responses in marine algae and bacteria, and a method measuring the cellular responses of specific classes of organic chemicals in sediment extracts. Multiple test methods were included in order to ensure that the test results were not unduly influenced by method-specific differences in contaminant sensitivity. The sampling plan was expanded relative to the SCBPP to include bays and harbors, where sediment contamination concentrations are usually highest.

- To compare the responses among multiple sediment toxicity test methods.

The concurrent use of multiple toxicity indicators provides the opportunity to examine their relative response to the same sample. Comparisons of the responses among indicators can be used to determine which methods are most sensitive to conditions in the SCB and whether each indicator is responding to the same or different sediment constituents.

- To evaluate the relationship between sediment toxicity and chemical contamination or changes in benthic communities throughout the SCB.

The relationship between sediment contamination, sediment toxicity, and benthic community response is still poorly understood. Relatively few data have been collected for the SCB where all three types of sediment quality measures have been utilized. Comparison of the sediment toxicity test results with chemistry and benthic community response provides the opportunity to “calibrate” toxicity test responses to impacts on communities and to determine if national sediment quality guidelines based upon chemical concentrations are applicable in the SCB.

Chapter II of this report describes the methods used to prepare the samples and measure toxicity. A quality assurance evaluation of the test results is provided in Chapter III, which addresses issues of data comparability and laboratory performance during the study. Chapter IV describes the test results for each of the indicators separately, illustrating patterns in the prevalence and severity of toxicity among the sampling strata. A comparison of the results for three of the tests is presented in Chapter V. Chapter VI uses the toxicity results to provide a regional assessment of sediment toxicity based upon the percent of area affected. This chapter also integrates the results from each indicator. Discussion of the results is contained in Chapter VII. Conclusions from the study are presented in Chapter VIII and recommendations for future studies are presented in Chapter IX.

Evaluation of the relationships between sediment toxicity, chemistry, and benthic community responses is not included in this report. These comparisons will be incorporated into a future Bight'98 integrative report, scheduled for completion in Fall 2001.

## II. METHODS

### A. Sampling Design

Three hundred and three sites on the continental shelf between Point Conception, California, and the United States-Mexico international border (Figure II-1) were sampled between July 13 and September 16, 1998. Sites were selected using a stratified random design. Five of the strata were located offshore (river mouths, large publicly owned treatment works [POTW] discharge areas, small POTW discharge areas, remaining shallow areas [5-30 m], and remaining mid-depth areas [30-120 m]). Three additional strata were located within bays and harbors, which included marinas, port/industrial areas, and other harbor areas (less-developed portions of bays and harbors that did not serve port/industrial or marina functions). In addition to the stratified random sites, 13 non-random sites were located in offshore areas, distant from known point sources. These 13 sites had been sampled at periodic intervals since 1977 (Thompson *et al.* 1987, Thompson *et al.* 1993, Word *et al.* 1979) and were selected for historical comparison. Results for the historical comparisons are not presented in this report.

Sites were selected randomly within each stratum, rather than by investigator pre-selection, to ensure that they were representative and could be extrapolated to the response of the entire stratum. Within the small POTW stratum, four samples were randomly placed in an approximately 3 km<sup>2</sup> area around each outfall. For the river mouth stratum, 2-4 random samples were placed within a 3 km radius of each river mouth, with the number of samples proportional to the area contained within 3 km (different areas resulted from the differing shape of the coastline near each outfall). For all other strata, a systematic component was added to the selection process to minimize clustering of sample sites. The systematic element was accomplished by using an extension of the sampling design used in the SCBPP and in the Environmental Protection Agency's (EPA's) Environmental Monitoring and Assessment Program (EMAP) (Stevens 1997). A hexagonal grid was placed over a map of the sampling area, a random subsample of hexagon cells was chosen from this population, and one sample was obtained at a randomly selected site within each grid cell. The hexagonal grid structure ensures systematic separation of the sampling effort, while the random selection of sites within grid cells ensures an unbiased estimate of ecological condition. Additional details of this site selection process are provided in the Coastal Ecology Study Plan (Bight'98 Steering Committee 1998).

Samples were collected for four types of toxicity measures: (1) amphipod survival, (2) QwikSed, (3) Microtox, and (4) P450 Human Reporter Gene System (HRGS). The number of sites sampled within each stratum (Table II-1) varied among measures because of limitations in laboratory capacity and available processing effort. The amphipod survival test was the primary indicator of toxicity in the study. Samples from 247 stations were selected for amphipod toxicity measurement, which included all bay/harbor stations and a subset of stations from the offshore stratum. Stations sampled for the QwikSed and Microtox indicators were a subset of those sampled for amphipod toxicity, with the primary emphasis on stations within bays and harbors (Table II-1). The largest number of stations was selected for the P450 HRGS, which shared the level of sampling effort used for sediment chemistry measurements.

## B. Field Methods

Sediment samples were collected with a 0.1 m<sup>2</sup> modified van Veen grab. Up to 2.5 L of sediment were collected for measurement of solid phase or elutriate toxicity using amphipod survival and QwikSed™ tests, respectively. A plastic (high-density polyethylene [HDPE], polycarbonate, or Teflon) scoop was used to collect sediment from the top 2 cm of the undisturbed surface material in the grab. Contact with sediment within 1 cm of the side of the grab was avoided in order to minimize cross-contamination. The sediment was placed in clean HDPE containers and distributed as follows: 2.25 L for amphipod test and 0.25 L for QwikSed™. Following collection, samples were stored on wet ice or refrigerated at 4° C.

Samples for the Microtox tests, which were based upon interstitial water analysis, were collected in the same manner, but were placed in a 0.25 L polycarbonate centrifuge bottle. These samples were also processed for acid volatile sulfide analysis, so the containers were filled completely, leaving no headspace. They were kept on wet ice or refrigerated at 4° C prior to centrifugation.

Organic solvent extracts for P450 HRGS analysis were obtained from surface sediment samples collected for hydrocarbon analysis. These samples were collected with a plastic, stainless steel, or Teflon-coated metal scoop and placed in a 0.25 L plastic or glass container with a Teflon-lined lid. The samples were initially placed on wet ice and then frozen within 24 h of collection.

## C. Laboratory Methods

Selection of sediment toxicity test methods was made by the Toxicology Committee, a technical committee comprised of representatives of the agencies participating in Bight'98. The Toxicology Committee also specified sample holding times and established performance criteria for each of the tests. Four laboratory indicators were used to evaluate sediment quality. Assessment of solid phase toxicity using the amphipod test was a cooperative effort involving seven laboratories (Table II-2). Each of the other indicators was analyzed by a single laboratory.

### Amphipod Survival

Toxicity to amphipods was determined using a 10-d survival test (U.S. EPA 1994) with *Eohaustorius estuarius*. Sediment samples were distributed among the test laboratories based upon the location of the laboratories. Amphipods and negative control sediment were collected from Beaver Creek, Oregon, a non-contaminated estuarine site, and held in the laboratory at least 4 d, but no longer than 14 d, prior to the test. Testing was conducted in 1 L glass test containers. Sediment was added to the test containers 1 d prior to the start of the test. Sediment samples were thoroughly mixed and then added to the test containers to form a sediment layer approximately 2 cm deep. Filtered seawater (20 g/kg salinity) was added slowly until a final volume of 800 mL was reached. Pipettes connected to an air source provided aeration. Sediments were allowed to equilibrate overnight. Each sample consisted of five randomly arranged replicates, along with an extra container for water quality. A negative control (amphipod collection site sediment) was included with each batch of samples tested.



At the start of the test, amphipods were added randomly until a total of 20 animals per container was present. Tests were conducted at 15° C under constant illumination. Test animals were exposed to the sediment samples for 10 d, with daily checks for air and mortality. Any floating animals were submerged by gently pushing them beneath the surface with a probe. At the end of the exposure period, the sediment was screened through a 0.5 mm screen and the number of surviving amphipods was recorded.

A laboratory intercalibration exercise was conducted prior to the start of testing in order to document the comparability of the results. This exercise demonstrated that comparable data were produced by each of the laboratories. Details of this exercise are presented in Appendix B.

A cadmium reference toxicity test was conducted concurrently with each sediment toxicity test. The reference toxicant test consisted of three replicates of five concentrations of dissolved cadmium, plus a control. No sediment was included in the reference toxicant tests. Ten amphipods were added to each replicate and exposed to the reference toxicant for 4 d. At the end of 4 d, the total number of surviving animals was recorded and the concentration causing 50% mortality (LC50) was calculated. The Spearman Karber method was used to calculate the LC50, which was then compared to a control chart prepared from the results of past reference toxicant tests. A test result within two standard deviations of the mean control chart LC50 was considered acceptable. A test not falling within two standard deviations was evaluated for acceptability by employing the best professional judgment of the laboratory.

Samples of overlying water and interstitial water were obtained from the extra test container for measurement of initial water quality (temperature, pH, dissolved oxygen, salinity, and total ammonia). Overlying water quality was also measured at the end of the exposure period. Water quality measurements made for the reference toxicant test were conducted using a similar methodology to the sediment phase of the test.

#### QwikSed Elutriate Test

The QwikSed elutriate test is a measure of light production from the bioluminescent dinoflagellate *Gonyaulax polyedra* following exposure to a sample. A reduction in bioluminescence relative to the control reflects an adverse effect of the sample on the test organisms.

QwikSed tests were conducted on sediment elutriates, which were prepared by mixing approximately 75 grams of wet sediment with 300 mL of filtered seawater. The mixture was stirred for 30 min and settled for 60 min; then the supernatant was poured off for use in the test (U. S. EPA 1991). All elutriates were passed through an 8 µm membrane filter prior to mixing for all dilutions. Five elutriate concentrations were prepared from the initial sediment-water mixture: 100, 50, 25, 12.5, and 6.25% (ASTM 1998). These dilutions were then mixed in 125 mL Erlenmeyer flasks with an equivalent volume of dinoflagellate cell stock to a final concentration of approximately 200 cells/mL. Dinoflagellates were obtained from laboratory cultures maintained in a filtered (0.45 µm) enriched seawater medium (ESM) under 40-watt

cool-white fluorescent bulbs on a 12:12 h (light:dark) cycle at 19° C. A control sample (ESM only) was included with each elutriate sample tested.

Three mL from each of the elutriate dilution flasks were pipetted into each of five disposable spectrophotometer cuvettes (five replicates per concentration). The cells were then incubated for 24 h at 19° C at a light intensity of 4,000 lux on a 12D:12L h photoperiod.

Total mechanical stimutable light (TMSL) was measured from each of the cuvettes after 24 h, using the QwikSed photometer system. The contents of the cuvette were stirred while in the photometer, stimulating the dinoflagellates to emit light that was detected by a photomultiplier tube (PMT). The PMT counts were accumulated during stirring and compared to the control response.

The salinity of the prepared elutriates was checked with a refractometer and maintained at ~33 g/kg. Total ammonia was measured with an Orion ammonia electrode; the temperature and pH of the elutriate were measured concurrently. Total ammonia was measured in all 100% elutriates and then converted to unionized ammonia. Unionized ammonia concentrations were then back-calculated for all dilutions in each sample.

Four reference toxicity tests with copper sulfate were conducted during the testing period. Two tests were conducted on August 25, 1998, a third test was conducted on September 16, 1998, and a fourth test was conducted on January 8, 1999. The copper sulfate reference toxicant data were examined to verify that all tests fell within  $\pm 2$  standard deviations of the mean. If outliers were observed, the test data were flagged to indicate that the health of the cells was questionable. Several toxicity tests were also conducted using ammonium chloride dissolved in seawater to determine the sensitivity of *G. polyedra* to ammonia.

#### Microtox Interstitial Water Test

Sediment interstitial (pore) water was assessed using the Microtox<sup>TM</sup> Rapid Toxicity Testing System, in which a change in light production from a luminescent bacterium relative to a control sample is used to measure toxicity. Interstitial water was obtained by centrifuging the sediment at 3,000 g for 20 min. The resulting interstitial water samples were stored frozen in borosilicate glass vials with Teflon-lined lids. The samples were analyzed using a Microtox M500 analyzer following the Microtox Comparison Test Method (Microbics Corp. 1995). Bacteria (*Vibrio fischeri* strain NRRL B-11177) were added to triplicate cuvettes containing 1.5 mL of 100% interstitial water and incubated at 15° C for 15 min. The luminescence of each replicate was measured and compared to a matching control (seawater) sample. The percent of difference in mean light production in the samples relative to the control sample was then calculated. Reproducibility of the test organism response was determined by testing a phenol reference toxicant solution at a single concentration.

#### P450 HRGS Solvent Extract Test

The P450 human reporter gene system (HRGS) test measures changes in luminescence of human hepatoma cells transfected with a luciferase reporter gene following exposure to solvent

extracts of sediment. An increase in luminescence indicates that the sample contains organic compounds that induce the production of cytochrome P450 (CYP1A1), such as PAHs, dioxins, furans, and coplanar PCBs (Anderson *et al.* 1995). Sediments from over 1,000 stations located in numerous U.S. coastal areas have already been tested by this method in NOAA investigations (Anderson *et al.* 1999).

The details of the HRGS method (EPA Method 4425) have been previously published (APHA 1998, ASTM 1999, EPA 1999); therefore, only a short summary follows. Hexane extracts of sediments were prepared by the Southern California Coastal Water Research Project (SCCWRP) and exchanged into dichloromethane (DCM) before testing. Extracts were applied at a volume of 2  $\mu$ L to single or duplicate wells in six-well plates containing 2 mL of culture media. After 16 h incubation, the cells were washed with Hank's Balanced Salt Solution (Mediatech, Herndon, VA) and lysed with 200  $\mu$ L of a solution containing 1% Triton, 25 mM Tricine, pH 7.8, 15 mM MgSO<sub>4</sub>, 4 mM EDTA, and 1 mM dithiothreitol (DTT). Cell lysates were centrifuged at 6,000 rpm for 10 s, and 50  $\mu$ L of the supernatant was applied to a 96-well plate, followed by addition of luciferase assay reagents. Luminescence in relative light units (RLUs) was measured using a ML2250 Luminometer (Dynatech Laboratories, Chantilly, VA) and divided by the RLUs measured for a solvent blank sample to determine the fold induction of the sample.

Extracts supplied by SCCWRP also included a method blank for each extraction batch, as well as extracts of the Santa Monica Bay (SMB) reference sediment used for interlaboratory calibration studies by the Bight'98 analytical chemistry laboratories. These samples were tested by HRGS concurrently with the sediment samples.

A reference toxicant, 1 ng/mL Tetrachlorodibenzo-*p*-dioxin (TCDD), was included with each test batch. The fold induction response of the TCDD was compared to a control chart; if a result deviated by more than two standard deviations from the running mean ( $106 \pm 17$ ), then the samples were reanalyzed. The HRGS fold induction response to all inducing compounds is linear to approximately 100, and then plateaus. For this reason, any sample producing a fold induction  $>100$  was diluted 1:10 and retested.

The HRGS results were reported in terms of benzo[a]pyrene equivalents on a dry weight basis ( $\mu$ g/g B[a]PEq). These values were calculated from the mean fold induction responses, based upon the HRGS concentration-response curve for benzo[a]pyrene, the amount of extract applied, the volume of the extract, and the sample dry weight.

#### **D. Data Analysis**

Data were analyzed using two methods: (1) Calculation of mean parameter response (e.g., average survival) in the SCB and in various strata (e.g., marinas), and (2) assessment of the fractional area within each stratum exceeding selected parameter thresholds.

Mean parameter values were calculated using a weighted mean:

$$m = \frac{\sum_{i=1}^n p_i w_i}{\sum_{i=1}^n w_i}$$

where:

$m$  = Mean value for population  $j$

$p_i$  = Parameter value (e.g., concentration) at station  $i$

$w_i$  = Weighting for station  $i$ , equal to the inverse of the inclusion probability for the site

$n$  = Number of stations sampled in population  $j$

Standard error of the mean response was calculated as:

$$\text{Standard Error} = \sqrt{\frac{n \sum_{i=1}^n ((p_i - m) * w_i)^2}{\left( \sum_{i=1}^n w_i \right)^2}}$$

Confidence intervals were calculated by multiplying the standard error by the two-tailed  $t$  value corresponding to  $\alpha = 0.05$ . Statistical differences between populations of interest were defined on the basis of non-overlapping confidence intervals. Use of the ratio estimator for the standard error approximates joint inclusion probabilities among samples and assumes a negligible spatial covariance, an assumption that appears warranted based upon preliminary examination of the data. The assumption is conservative in that its violation would lead to an overestimation of the confidence interval (Stevens and Kincaid 1997).

The percent of area exceeding a selected threshold was calculated after converting the data to its binomial form. For any sample observation,  $p_i$  was 1 if it exceeded the threshold value and 0 otherwise. The proportion of area that exceeded the selected threshold value was taken as the weighted mean of the indicator variable  $p_i$ . The toxicity thresholds used were specific to each test and are described below.

For the amphipod test, two events had to occur before a sample could be classified as toxic: (1) a statistically significant reduction in survival compared to the control and (2) a minimum percent reduction in survival between the sample and control. Statistical significance was determined by performing a  $t$ -test between the sample and control with a 0.05 level of significance. Samples with a significant reduction in mean survival that was greater than 20% relative to the control (i.e., a control normalized survival of less than 80%) were determined to be moderately toxic. This measure of toxicity has been used in previous surveys (Bay *et al.* 1998, U. S. EPA 1994) and has been shown to represent a 90% power to determine statistical

significance in survival between control and sample (Thursby *et al.* 1997). Samples with mean survival rates of less than 50% of the control were classified as highly toxic.

The QwikSed test responses were corrected for the influence of ammonia toxicity by using the dose-response relationship calculated from experiments using seawater spiked with known concentrations of ammonia. A nonlinear logistic regression was used to describe the influence of unionized ammonia on QwikSed luminescence. The resulting best-fit equation was:

$$y = a_0 + \frac{a_1}{\left(1 + (x/a_2)^{a_3}\right)}$$

where:

$y$  = QwikSed luminescence

$x$  = Unionized ammonia concentration

$a_0 = -402.553$

$a_1 = 517.485$

$a_2 = 2.737$

$a_3 = 0.706$

The QwikSed data (expressed as a percent of the control luminescence) were corrected for ammonia toxicity using a two-step process. First, the net change in luminescence due to ammonia ( $y_a$ ) was determined by subtracting the predicted luminescence ( $y$ ) from the control ( $y_c = 100 - y$ ). Second, the corrected value ( $y_c$ ) was obtained by increasing the measured luminescence ( $y_m$ ) by the amount due to ammonia ( $y_c = y_m + y_a$ ).

Example calculation:

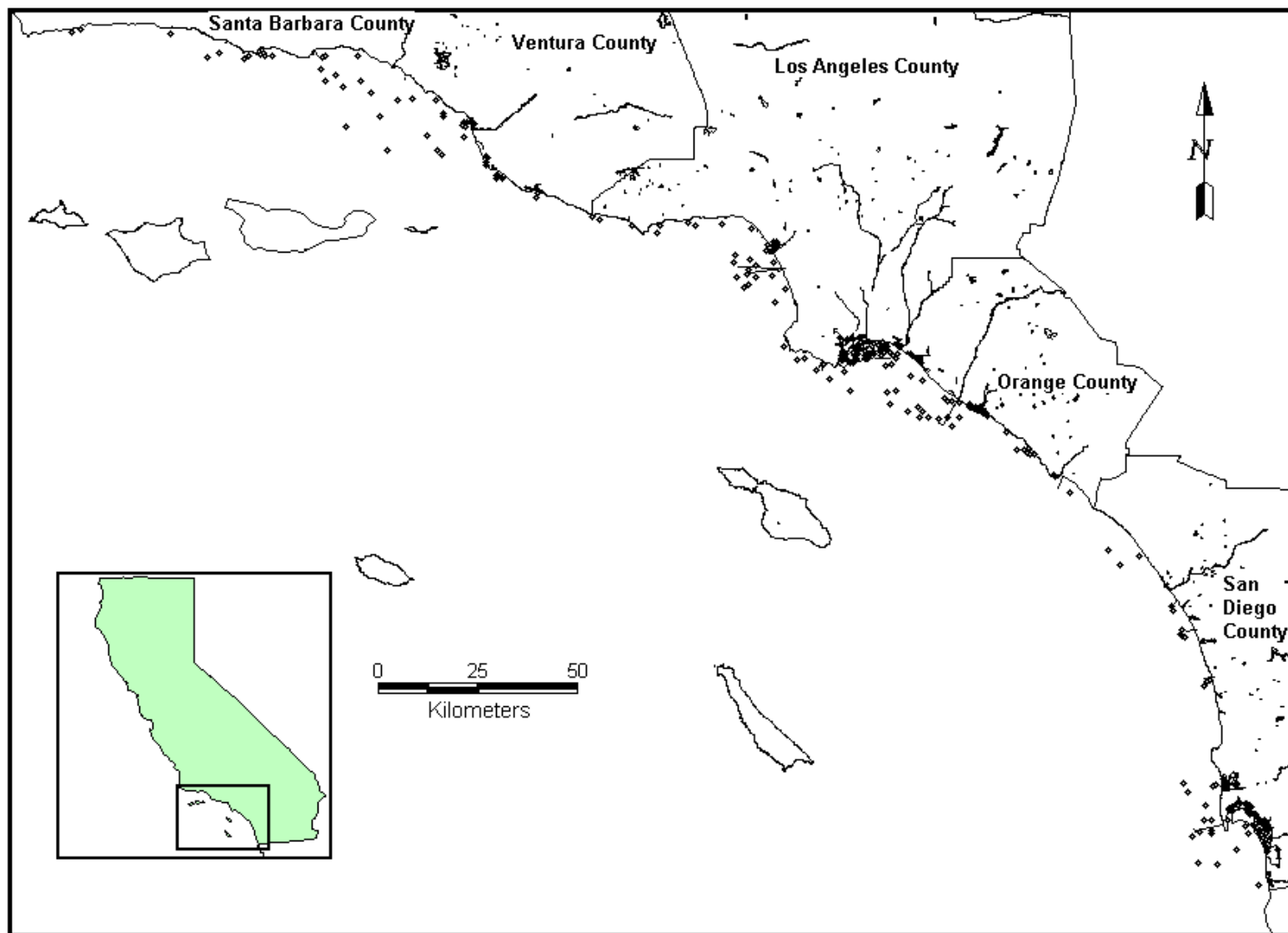
If: Measured luminescence ( $y_m$ ) = 70.2% of control  
Unionized ammonia ( $x$ ) = 0.0527 mg/L

Then: Predicted luminescence due to ammonia ( $y$ ) = 85.0  
Net change due to ammonia ( $y_a$ ) =  $100.0 - 85.0 = 15.0$   
Corrected value ( $y_c$ ) =  $70.2 + 15.0 = 85.2\%$

Samples with <84% of control bioluminescence were classified as being moderately toxic. This value represents the level at which there is a 90% power to detect a significant difference in response from the control. Highly toxic samples were defined as those with <50% of control bioluminescence.

For the Microtox test, samples were classified as toxic if there was a 5% reduction in luminescence relative to the control. This value corresponds to the level at which there is a 90% power to detect a significant difference in response relative to the control. Samples producing <50% of the control luminescence were classified as highly toxic.

Two thresholds were used for the classification of the P450 HRGS test responses. Samples containing  $\geq 32$   $\mu\text{g/g}$  B[a]PEq were classified as potentially toxic; this value corresponds to the upper 99% confidence interval of samples analyzed in previous surveys (Anderson *et al.* 1999). Values less than 11  $\mu\text{g/g}$  B[a]PEq are not likely to be associated with any adverse biological effects, but impacts on organisms are unknown at concentrations between 11 and 32  $\mu\text{g/g}$  B[a]PEq. The classification of highly toxic was applied to samples with  $\geq 60$   $\mu\text{g/g}$  B[a]PEq, a level associated with the occurrence of degraded benthic communities (Fairey *et al.* 1996).



**FIGURE II-1. Locations of all stations sampled for sediment toxicity during the Bight'98 project.**

**TABLE II-1. Number of stations selected for toxicity evaluation for each stratum.**

Stratum	Number of Stations per Indicator Type			
	Amphipod	QwikSed	Microtox	P450 HRGS
<b>Offshore</b>				
River Mouths	44	1	1	44
Shallow	30	12	12	30
Mid-depth	30	17	17	30
POTW	30	30	30	66
<b>Bays/Harbors</b>				
Marinas	39	39	39	39
Ports/Industrial	35	35	35	35
Other	39	39	39	39
<b>Historically Sampled</b>				
Shallow				6
Mid-depth				7
<b>Total</b>	<b>247</b>	<b>173</b>	<b>173</b>	<b>296</b>



**TABLE II-2. List of participating laboratories and toxicity indicators measured.**  
**A=Amphipod test, M=Mictotox test, Q=QwikSed test, P=P450 HRGS test.**

	A	M	Q	P
Aquatic Bioassay and Consulting Laboratories	√			
City of Los Angeles (EMD)	√			
City of San Diego (Metro)	√			
Columbia Analytical Services				√
Marine Pollution Studies Laboratory	√			
MEC Analytical Systems	√			
Orange County Sanitation District (OCSD)	√	√		
SCCWRP	√			
U.S. Navy (SPAWAR)			√	

### III. QUALITY ASSURANCE EVALUATION

#### A. Sampling Success

Bight'98 field crews maintained a good sampling record. Samples were successfully collected at 92-100% of the total sites planned for each toxicity indicator (Table III-1). For some strata, the number of samples collected exceeded the nominal number planned; this situation resulted from the inclusion of 10-20% more stations in the sampling plan in order to compensate for the inability to collect samples at some stations. Shallow and mid-depth offshore locations were nearly all collected successfully. The lowest completion rate was near river mouths and in areas of San Diego Bay, where the presence of coarse sediments hindered grab operations.

**TABLE III-1. Bight'98 toxicity sample collection success.**

Strata	Amphipod		QwikSed & Microtox		P450 HRGS	
	Number Sampled	Percent of Target	Number Sampled	Percent of Target	Number Sampled	Percent of Target
<b>Offshore</b>						
River Mouths	31	70	1	100	31	70
Shallow	33	100	13	100	33	97
Mid-depth	34	100	21	100	34	100
POTW	30	100	30	100	48	73
<b>Bays/Harbors</b>						
Marina	39	100	39	100	39	100
Ports/Industrial	37	100	37	100	37	100
Other	37	95	37	95	37	95
<b>Historically Sampled</b>					13	100
<b>TOTAL</b>	241	98	178	100	272	92

#### B. Sample Storage

An objective of 14 d of storage time between collection and test initiation was established for the amphipod and QwikSed tests. This guideline was met for 89% of the amphipod test samples (Table III-2). Samples for QwikSed were usually stored for a longer period of time. Only 31% of the QwikSed samples were tested within two weeks, but all of the samples were tested within two months. Delays in testing were due to two factors: (1) the rapid collection of sediment samples by multiple agencies, which overwhelmed laboratory capacity; and (2) the need to retest some QwikSed samples that failed test performance criteria. The impact of extended sample storage time on the QwikSed results has not been documented, but analysis of the data indicates that the effects, if any, were minor.

Microtox analysis of interstitial water samples was completed within the six-month interval established by the Toxicology Committee. An extended holding time was needed for this test in order to accommodate other laboratory activities. The planned storage conditions were not maintained for these water samples, however. Interstitial water samples were held under refrigeration after extraction from the sediments, instead of being frozen, for approximately four months until they were transported to the testing laboratory. The samples were then frozen for up to two months and then thawed for analysis. A post-survey study conducted to evaluate the effect of extended sample storage indicated that prolonged storage under refrigeration produced significant and unpredictable changes in Microtox results (Appendix C). The Microtox data were therefore declared invalid and were not used in assessing sediment toxicity.

Sediment samples for HRGS testing were stored frozen for approximately 12 months before extraction. While this period is longer than the 6 months specified by the Toxicology Committee, the types of compounds detected by this method (high molecular weight PAHs, coplanar PCBs, dioxins, furans) have demonstrated stability under frozen conditions. A sample storage study conducted during Bight'98 indicated that concentrations were stable during the storage period. The HRGS tests were completed and the data were reported within three months of extraction, within the objective specified in the Bight'98 Quality Assurance Plan.

**TABLE III-2. Bight'98 toxicity sample holding time.**

Time	Amphipod		QwikSed		Microtox		P450 HRGS	
	Number	Percent	Number	Percent	Number	Percent	Number	Percent
≤ 2 weeks	214	89	55	31				
3 weeks	27	11	40	23				
2 months			83	46				
6 months					86	100		
9-12 months							272	100

### C. Test Performance

#### Amphipod Survival

All of the 241 samples collected were successfully tested. The mean control survival rate was 96% (values ranged from 75-100%).

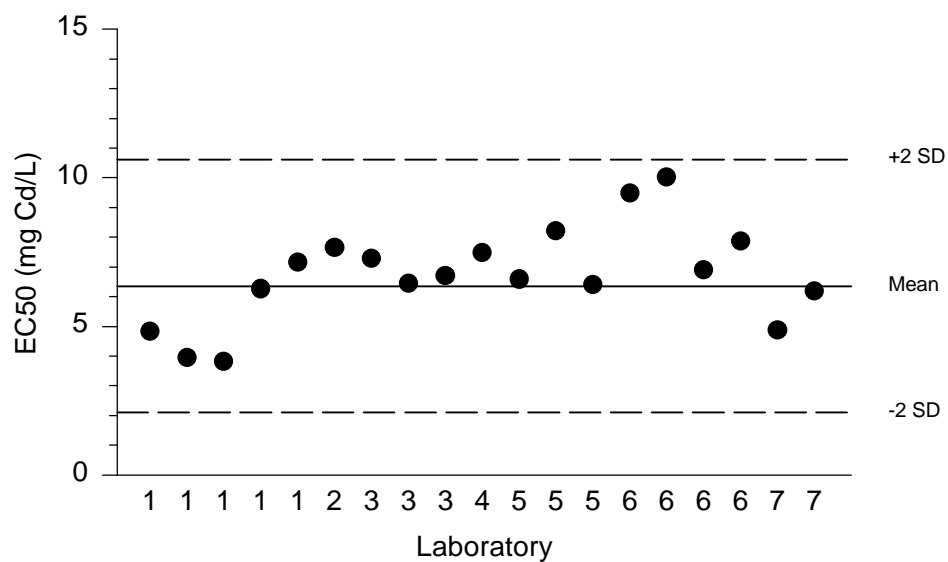
All but two of the amphipod sediment toxicity tests met all necessary acceptability criteria. The two cases where test performance criteria were not met were judged to have little influence on the outcome of the test. In one case, one control replicate had a percent survival of 75%, less than the 80% minimum specified in the protocol. In the other case, the experiment was terminated 1 d early (after 9 d). Examination of the results for both of these tests indicated that the outcome of the test was unlikely to have changed had all of the test criteria been met. Consequently, these tests were not repeated and the data were used in this report.

Water quality parameters (pH, dissolved oxygen [DO], salinity, and ammonia) were generally within acceptable limits (U.S. EPA 1994). The desired range for salinity was  $20 \pm 3$  g/kg. Salinity in the overlying water ranged from 18-27 g/kg during the Bight'98 tests. Interstitial water salinity ranged from 17-35 g/kg. Although above the desired limits, the test salinities were within the tolerance range of *E. estuarius* (1-35 g/kg). Temperatures in overlying water ranged from 13.5-16.7° C and were within the desired range of  $15 \pm 3^\circ$  C. In overlying water, pH ranged from 7.01-9.63, while DO ranged from 4.4-10.8 mg/L. Interstitial water pH and DO ranged from 6.79-8.20 and 3.9-8.2, respectively.

The concentration of unionized ammonia in the overlying water ranged from 0.00 to 0.49 mg/L, except for a single value of 1.33 mg/L that exceeded the ammonia acceptability criterion of 0.8 mg/L. The high value was measured at the end of the experiment, so no corrective action could be taken.

A total of 19 reference toxicant tests with cadmium were conducted by the laboratories during the survey. The purpose of reference toxicant tests is to determine whether test organism

response and test procedures are comparable among different testing periods and laboratories. Reference toxicant test results indicated that the laboratory test organisms and individual laboratory performances were similar. The mean LC50 values from participating laboratories ranged from 5.20-8.56 mg/L (with a mean value of 6.74). Control survival ranged from 90-99% (with a mean survival rate of 96%). All laboratories submitted acceptable reference toxicant results and all values were within two standard deviations of the calculated mean LC50 (Figure III-1).



**FIGURE III-1. Summary of cadmium reference toxicant test results for amphipod survival tests. Dashed lines indicate the value corresponding to two standard deviations from the mean.**

## QwikSed

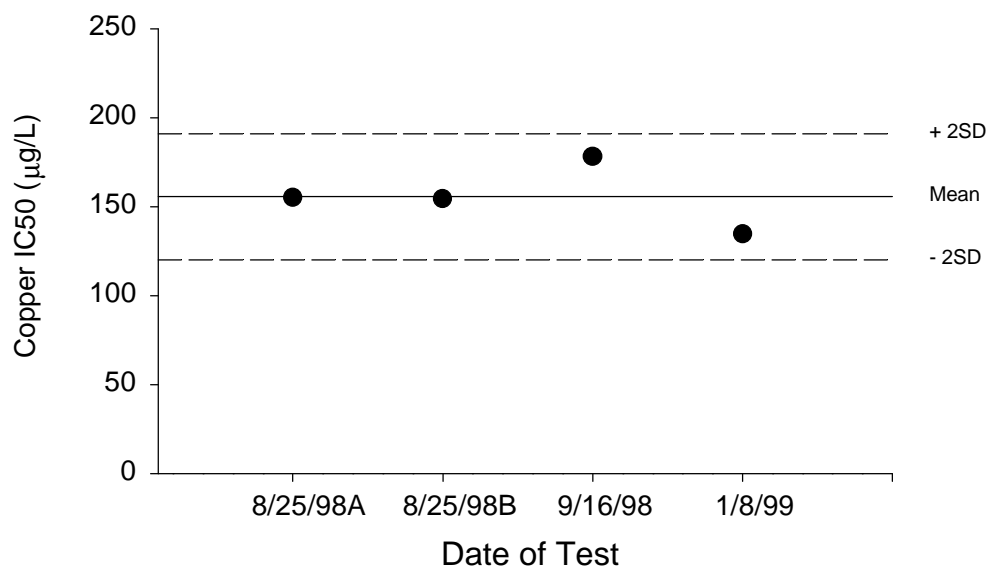
Most toxicity tests met the acceptability requirements established for the respective test. Out of a total of 178 sediment samples collected, 173 met the appropriate American Society for Testing and Materials (ASTM) 1998 criteria (ASTM 1998). The failed tests had poor survival of the dinoflagellates in the control samples. Additional tests showing poor control performance (less than 1 million counts per 30 seconds) were re-run several days later. A total of 53 samples were retested either because of low controls and/or poor test precision.

A copper sulfate reference toxicant was analyzed three times during the testing period and once after completion of the analyses. All test results fell within two standard deviations of a mean IC50 of 156 ug/L (Figure III-2). These results indicate that the sensitivity of the laboratory culture of *G. polyedra* did not change throughout the sediment test period.

Elutriate pH, temperature, and salinity were within acceptable ranges for all tests. Temperature was maintained at  $19 \pm 1^{\circ} \text{C}$  and the pH of 100% elutriates ranged from 7.32-8.74. Salinity ranged from 32-35 g/kg.

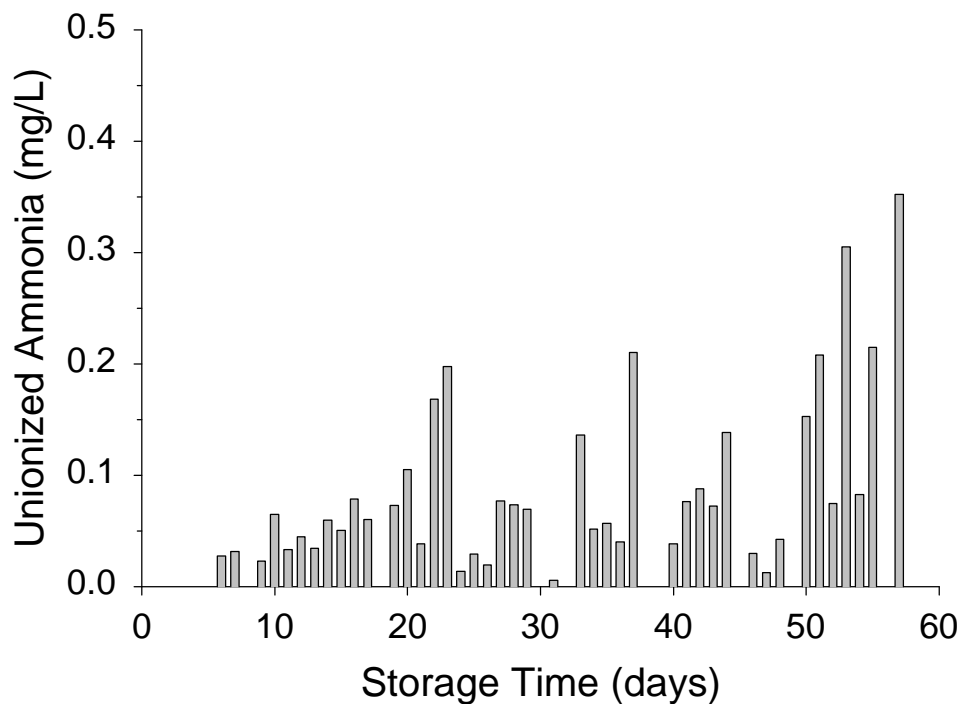
An increase in elutriate ammonia concentration with storage time was observed (Figure III-3). Samples stored longer than 30 d tended to have higher ammonia concentrations. Most of the 100% elutriate samples contained unionized ammonia concentrations above 0.04 mg/L, the level associated with toxic effects (Figure III-4). Two actions were taken to minimize the influence of ammonia toxicity on the results. First, data from only the 25% elutriate concentration, which contained one-fourth of the ammonia concentration, were evaluated in the study. In addition, all data were corrected for ammonia toxicity by increasing the luminescence value by the amount that was lost due to ammonia. The regression procedure used to perform the correction is described in Section II (Methods).

Correction for ammonia toxicity had a minor impact on most of the data for the 25% elutriate. Little or no change was observed for 80% of the samples (Figure III-5). However, correction increased the measured value by more than 10 percentage points in 16 stations (approximately 10% of the stations tested). Fourteen stations were reclassified as a result of the correction, either from moderately toxic to nontoxic or from highly toxic to moderately toxic. Most of the reclassified stations were located within bays and harbors (Figure III-6); eight of the reclassified stations were located in San Diego Bay.

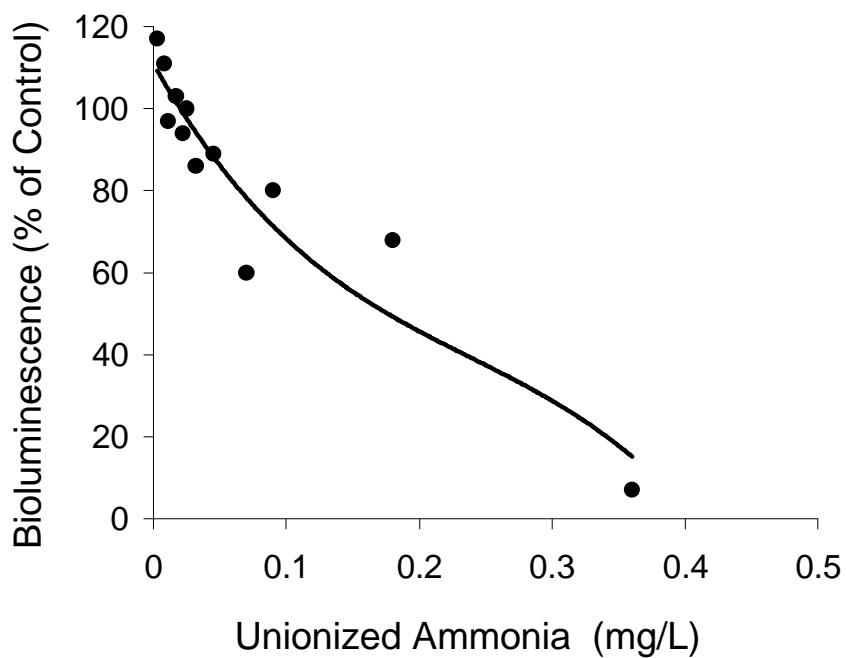


**FIGURE III-2. QwikSed reference toxicant results. Symbols represent copper reference toxicant samples analyzed during or after batches of Bight'98 samples. Dashed lines indicate the value corresponding to two standard deviations from the mean.**

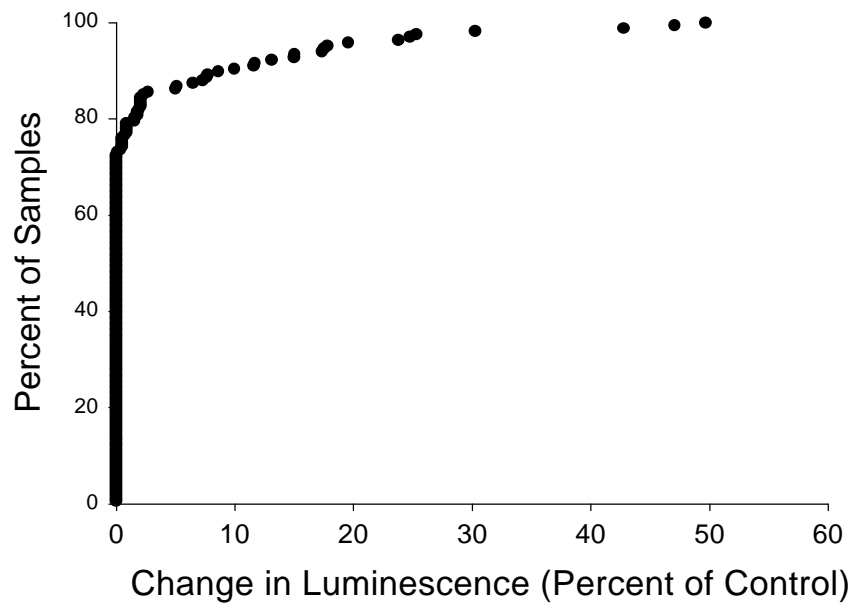




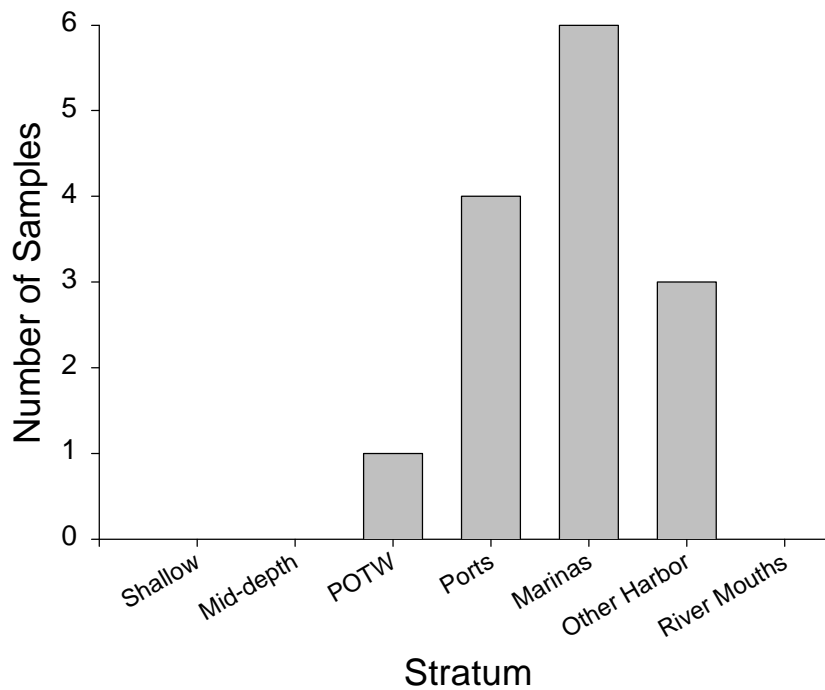
**FIGURE III-3. Concentration of ammonia in elutriate samples stored prior to QwikSed analysis. Values are the mean of all samples stored for the indicated number of days.**



**FIGURE III-4. Dose-response plot of ammonia effects on *G. polyedra* with regression curve.**



**FIGURE III-5. Influence of ammonia correction procedure on QwikSed luminescence value.**



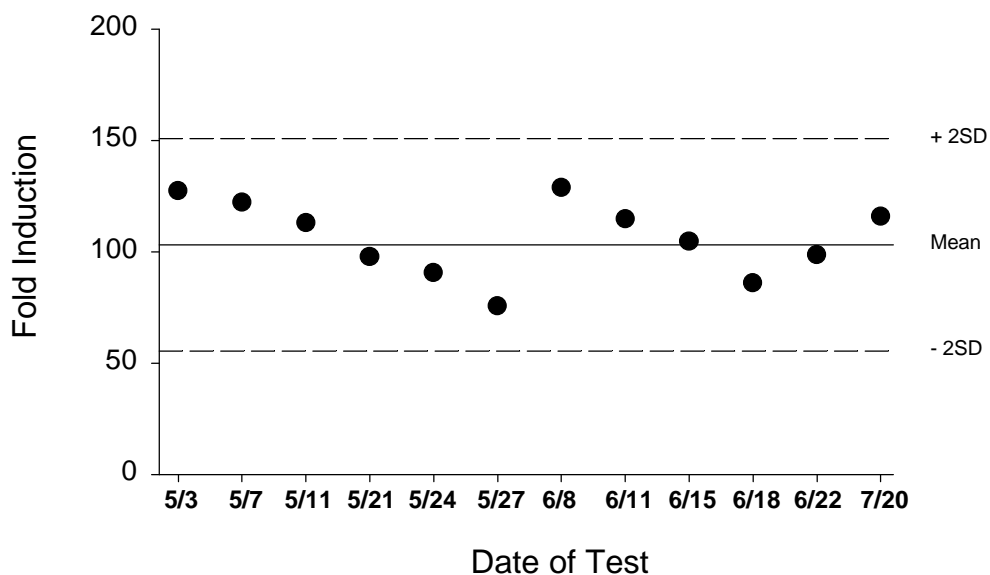
**FIGURE III-6. Reclassification of stations resulting from correction of QwikSed results for ammonia toxicity. All changes reflected a reduction in the severity of effect.**

### P450 HRGS

The HRGS assay was performed on 12 different days. The mean response to the reference inducer, TCDD, was a fold induction of 106.3 with a coefficient of variation of 16% (Figure III-7). This response was always within the control chart acceptable limits of 55-151, based upon prior data. The coefficient of variation among duplicate sediment extracts was always less than 10%.

In three cases, (Stations 2263, 2251, and 2360) the HRGS fold induction response to the samples was higher than 100, which was beyond the linear range of the assay. These samples were diluted 1:10 in DCM and retested on the following test date. The fold induction response to the diluted sample was acceptable (less than 100) in each case and the data were included in the database.

Five samples of the SMB reference material were tested to examine the combined variability of the extraction and test procedure. Results for four of the samples had low variability, with HRGS responses of 96-170  $\mu\text{g B[a]PEq/g}$  (Table III-3). The HRGS results were approximately three times higher for the last sample tested (387  $\mu\text{g B[a]PEq/g}$ ). Since the reference toxicant results (Figure III-7) indicated that the assay was performing normally during analysis of the last SMB extract, it is assumed that non-homogeneity of the sediment was the cause of this high variation.



**FIGURE III-7. Response of the P450 HRGS assay to the reference inducer (TCDD) over the testing period for Southern California Bight'98 samples. Dashed lines indicate the value corresponding to two standard deviations from the mean. All tests were conducted in 1999.**

**TABLE III-3. The HRGS assay response to Santa Monica Bay reference sediment.**

Analysis Date	Dilution	B[a]PEq (µg/g)
3-31-99	1:1	104.4
4-7-99	1:10	95.8
4-9-99	1:1	169.5
4-13-99	1:1	139.9
4-19-99	1:10	386.7
Mean		179.3
Standard Deviation		119.6

## IV. DESCRIPTIVE RESULTS

### A. Amphipod Survival

Among 241 sediment samples collected throughout the SCB, 42 (17% of the total) showed some degree of toxicity (Table IV-1, Figure IV-1). Eight samples (3%) were classified as highly toxic (survival less than 50% of the control value), while 34 samples (14%) were classified as moderately toxic (survival less than 80% of the control).

Amphipod survival was closely related to proximity to bays and harbors, where 25% of the samples were classified as toxic. All of the highly toxic sediments detected in this study were collected from within harbors, or rivers that drain into harbors (i.e., the Los Angeles River). Within harbors (Table IV-1), samples from marinas had the highest frequency of toxicity (38%). Samples from port and industrial areas were toxic in 22% of the cases. Finally, 14% of samples collected from other areas of bays and harbors were toxic to amphipods. For many harbors, samples collected in inner areas were more toxic than those collected near entrances (Appendix E).

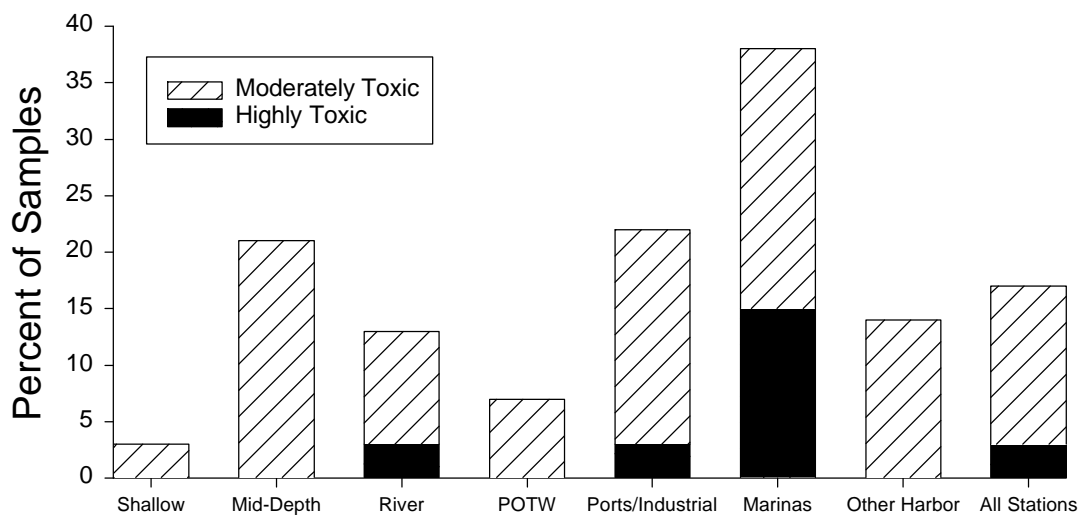
The shallow stratum had the lowest percentage of toxic samples (3%). Samples from POTW outfall areas were toxic in 6% of the cases, while 13% of samples taken near river mouths produced a toxic response. The mid-depth stratum had the highest frequency of toxicity among the offshore strata, with 21% of sites showing moderate toxicity to amphipods.

Among individual harbors (Table IV-2), the largest number of highly toxic samples (5) came from Newport Bay, and the largest number of moderately toxic samples (9) came from San Pedro Bay (including the Los Angeles River). Newport Bay was the only harbor in which toxic samples exceeded nontoxic samples (9 toxic versus 2 nontoxic, or 82% toxicity). No toxicity was found in Dana Point Harbor, Mission Bay, or Ventura Harbor, although sampling was less extensive in these areas (1-3 samples each). San Diego Bay sediments also showed relatively infrequent toxicity (11%).

Since the numbers of toxic versus nontoxic samples do not indicate the magnitude of toxicity among samples, comparisons of strata by the mean percent of controls is also presented for each toxicity classification (Table IV-1). For highly toxic samples, one sample from the Los Angeles River produced the lowest survival rate (7% of the control), followed by one sample from a ports/industrial area (16%), followed by the average of six marina samples (29%). Among moderately toxic samples, mean percents of controls were similar (57-73%), as were the nontoxic samples (91-98%). Within the SCB as a whole, the mean percent of control for highly toxic sediments was 22%, for moderately toxic sediments 68%, and for nontoxic sediments 93%.

**TABLE IV-1. Sediment samples toxic to amphipods from seven strata in the Southern California Bight.**

	Highly Toxic				Moderately Toxic				Nontoxic			
	No.	%	Mean	95% CI	No.	%	Mean	95% CI	No.	%	Mean	95% CI
<u>Offshore</u>												
Shallow	0	0	na	na	1	3	68	na	32	97	98	96-99
Mid-Depth	0	0	na	na	7	21	73	71-76	27	79	93	90-96
River Mouths	1	3	7	na	3	10	64	60-67	27	87	96	94-98
POTW Outfall Areas	0	0	na	na	2	7	57	na	28	93	97	95-100
<u>Bays/Harbors</u>												
Ports/Industrial	1	3	16	na	7	19	73	67-80	29	78	90	87-92
Marinas	6	15	29	18-41	9	23	68	61-74	24	62	94	90-98
Other	0	0	na	na	5	14	65	54-76	32	86	91	89-94
All Stations	8	3	22	16-28	34	14	68	66-71	199	83	93	92-94



**FIGURE IV-1. Percent of sediment samples toxic to amphipods from seven strata in the Southern California Bight.**

**TABLE IV-2. Sediment samples toxic to amphipods from nine harbors and bays in the Southern California Bight. San Pedro Bay includes stations located near the Los Angeles River and the Terminal Island Wastewater Treatment Plant.**

	Highly Toxic		Moderately Toxic		Nontoxic	
	Number	Percent	Number	Percent	Number	Percent
Ventura Harbor	0	0	0	0	1	100
Channel Islands Harbor	1	33	1	33	1	33
Marina del Rey	0	0	3	43	4	57
San Pedro Bay	2	4	9	20	34	76
Anaheim Bay	0	0	1	33	2	67
Newport Bay	5	45	4	36	2	18
Mission Bay	0	0	0	0	3	100
Dana Point Harbor	0	0	0	0	3	100
San Diego Bay	0	0	5	11	41	89
All Harbors	8	7	23	19	91	74

## B. QwikSed

Results for the 25% elutriate concentration were used to describe the magnitude and spatial patterns of toxicity to *G. polyedra*. This concentration was selected in order to provide a conservative measure of toxicity that would minimize the effects of interferences from noncontaminant factors such as ammonia. Toxicity results were subdivided into three categories: highly toxic (<50% of controls), moderately toxic (51-83% of controls), and nontoxic (>84% of controls).

Out of a total of 173 samples tested, 37 samples (20%) were classified as toxic (11 highly toxic and 26 moderately toxic). All toxic samples were located within POTW or bay/harbor strata. Seventy-nine percent of all samples tested were classified as nontoxic (Table IV-3).

Four (14%) of the samples from the POTW stratum were classified as highly toxic (Figure IV-2). No toxic samples were present within the shallow and mid-depth strata, which included many of the stations offshore of Ventura and Santa Barbara counties that were found to be toxic to amphipods (Appendix D).

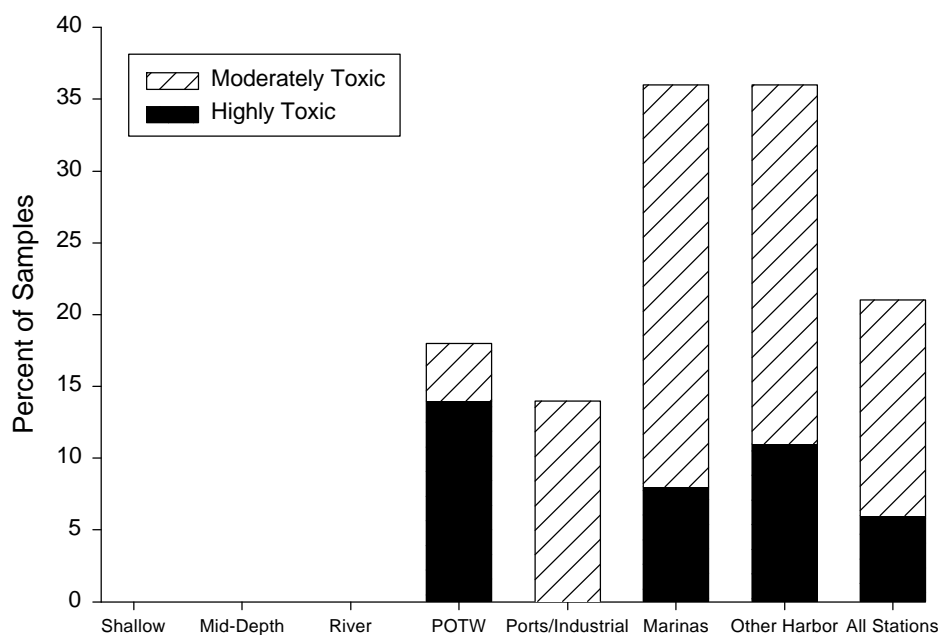
Of 116 sediment samples collected from bays/harbors, 7 samples (6%) were classified as highly toxic and another 25 samples (22%) were classified as moderately toxic. The most prevalent stratum of toxicity was marina areas, where 36% of the stations were classified as moderately or highly toxic. The QwikSed test classified 84% of all harbor/bay samples from the SCB as nontoxic (Table IV-4).

Within individual harbors, 1 out of 7 samples in Marina del Rey was classified as highly toxic, while 1 out of 42 samples in San Pedro Bay, 2 out of 11 samples in Newport Bay, and 3 out of 43 samples in San Diego Bay were also classified as highly toxic (Table IV-4). Forty-five percent of the samples (5 out of 11) collected in Newport Bay and 28% of the samples (13 out of 43) collected in San Diego Bay were moderately toxic.



**TABLE IV-3. Sediment samples toxic to QwikSed from seven strata in the Southern California Bight.**

	Highly Toxic				Moderately Toxic				Nontoxic			
	No.	%	Mean	95% CI	No.	%	Mean	95% CI	No.	%	Mean	95% CI
<u>Offshore</u>												
Shallow	0	0	na	na	0	0	na	na	13	100	98	95-101
Mid-Depth	0	0	na	na	0	0	na	na	21	100	98	97-100
River Mouths	0	0	na	na	0	0	na	na	1	100	100	na
POTW Outfall Areas	4	14	7	0-21	1	4	51	na	23	82	98	96-100
<u>Bays/Harbors</u>												
Ports/Industrial	0	0	na	na	5	14	79	76-82	30	86	97	95-99
Marinas	3	8	39	20-60	11	28	76	73-81	25	64	98	96-99
Other	4	11	29	5-54	9	25	66	60-73	23	64	98	96-99
All Stations	11	6	11	0-22	26	15	64	55-74	136	79	98	97-100



**FIGURE IV-2. Percent of sediment samples toxic to QwikSed from seven strata in the Southern California Bight.**

**TABLE IV-4. Sediment samples toxic to QwikSed from nine harbors and bays in the Southern California Bight. San Pedro Bay includes stations located near the Los Angeles River and the Terminal Island Wastewater Treatment Plant.**

	Highly Toxic		Moderately Toxic		Nontoxic	
	Number	Percent	Number	Percent	Number	Percent
Ventura Harbor	0	0	0	0	1	100
Channel Islands Harbor	0	0	1	33	2	67
Marina del Rey	1	14	0	0	6	86
San Pedro Bay	1	2	4	10	37	88
Anaheim Bay	0	0	1	33	2	67
Newport Bay	2	18	5	45	4	36
Mission Bay	0	0	1	33	2	67
Dana Point Harbor	0	0	0	0	3	100
San Diego Bay	3	7	13	30	27	63
All Harbors	7	6	25	22	84	72

### C. P450 HRGS

The HRGS assay identified markedly elevated concentrations of CYP1A1-inducing compounds (PAHs, coplanar PCBs, dioxins, and furans) in 30 of 259 samples (11%) tested from the seven strata (Table IV-5). Responses in 14 of the toxic samples were classified as highly toxic ( $\geq 60$   $\mu\text{g/g}$  B[a]PEq), while the remainder contained 32-59  $\mu\text{g/g}$  B[a]PEq and were classified as potentially toxic. From previous NOAA studies (Anderson *et al.* 1999), it appears that sediments producing a response of 11  $\mu\text{g/g}$  B[a]PEq or less would not cause biological effects, but insufficient data were found to determine whether levels between 11 and 32  $\mu\text{g/g}$  B[a]PEq would produce any impacts on the biota.

Few of the toxic stations were located in the offshore or river mouth strata. Potential toxicity was detected in 4 samples (8%) from POTW outfall areas. Toxicity was detected in 1-3 stations from each of the other offshore strata. A very high response (903  $\mu\text{g/g}$  B[a]PEq) was measured in 1 sample, located near Coal Oil Point in Santa Barbara County. This response was more than four times greater than the highest concentrations measured in bay/harbor area samples ( $\leq 182$   $\mu\text{g/g}$  B[a]PEq).

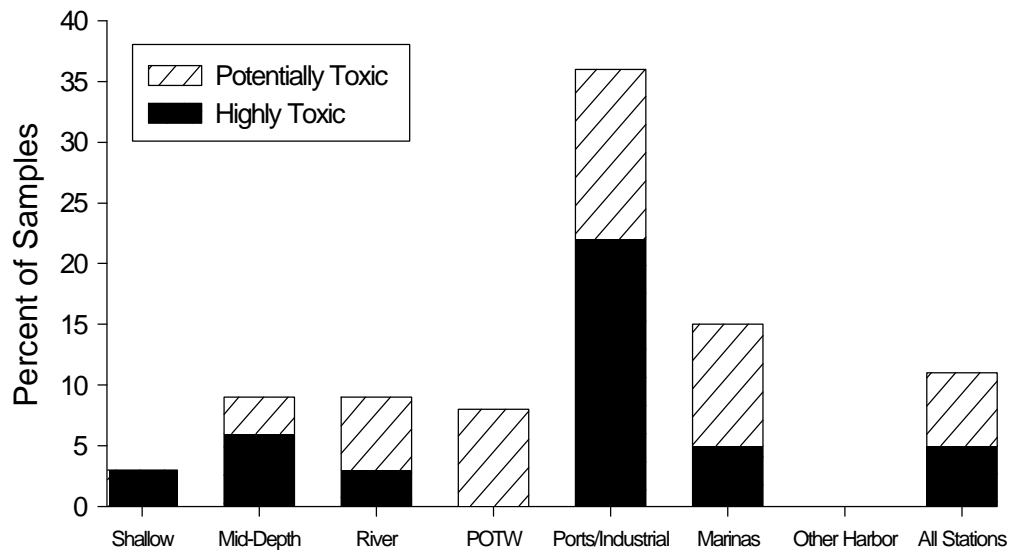
Ports/industrial areas contained the largest number of toxic stations; 36% of the stations within this stratum were classified as toxic (Figure IV-3), with the majority of these stations classified as highly toxic. A lower frequency of toxicity was detected in marina areas (15%) and no samples from other locations within bays/harbors contained toxic concentrations of inducing compounds.

Aside from the extremely high concentration in the Coal Oil Point sample, the largest individual HRGS responses were measured in samples from port/industrial areas in San Pedro Bay and San Diego Bay. Mean concentrations of HRGS-inducing compounds in each classification were not significantly different among strata (Table IV-5). Stations classified as highly toxic in each stratum contained an average of 88-115  $\mu\text{g/g}$  B[a]PEq, while potentially toxic stations contained an average of 35-52  $\mu\text{g/g}$  B[a]PEq.

Toxic samples were located in five of nine individual bays or harbors (Table IV-6). San Diego Bay contained the largest number of toxic stations, with 11 of 46 (24%) stations from this bay being classified as potentially or highly toxic. Relatively high percentages of toxic stations were also identified for Marina del Rey (43% of 7 stations) and San Pedro Bay (16% of 45 stations). Only 1 of the 11 stations in Newport Bay was found to be toxic.

**TABLE IV-5. Sediment samples toxic to HRGS from seven strata in the Southern California Bight.**

	Highly Toxic				Potentially Toxic				Nontoxic			
	N	%	Mean	95% CI	N	%	Mean	95% CI	N	%	Mean	95% CI
<u>Offshore</u>												
Shallow	1	3	90	na	0	0	na	na	32	97	4	3-5
Mid-Depth	2	6	490	na	1	3	35	na	31	91	8	6-10
River Mouths	1	3	88	na	2	6	44	na	28	90	4	3-6
POTW Outfall Areas	0	0	na	na	4	8	53	47-61	44	92	8	6-9
<u>Bays/Harbors</u>												
Ports/Industrial	8	22	112	80-144	5	14	42	30-54	24	65	17	16-21
Marinas	2	5	90	na	4	10	43	32-55	33	85	12	10-14
Other	0	0	na	na	0	0	na	na	37	100	10	9-12
All Stations	14	5	392	0-897	16	6	39	32-46	229	88	7	5-8



**FIGURE IV-3. Percent of sediment samples toxic to HRGS from seven strata in the Southern California Bight.**

**TABLE IV-6. Sediment samples toxic to HRGS from nine harbors and bays in the Southern California Bight. San Pedro Bay includes stations located near the Los Angeles River and the Terminal Island Wastewater Treatment Plant.**

	Highly Toxic		Potentially Toxic		Nontoxic	
	No.	Percent	No.	Percent	No.	Percent
Ventura Harbor	0	0	0	0	1	100
Channel Islands Harbor	0	0	0	0	3	100
Marina del Rey	1	14	2	29	4	57
San Pedro Bay	3	7	4	9	38	84
Anaheim Bay	0	0	0	0	3	100
Newport Bay	1	9	0	0	10	91
Mission Bay	0	0	0	0	3	100
Dana Point Harbor	0	0	0	0	3	100
San Diego Bay	6	13	5	11	35	76
All Harbors	11	9	11	9	100	82

## V. COMPARISON OF INDICATORS

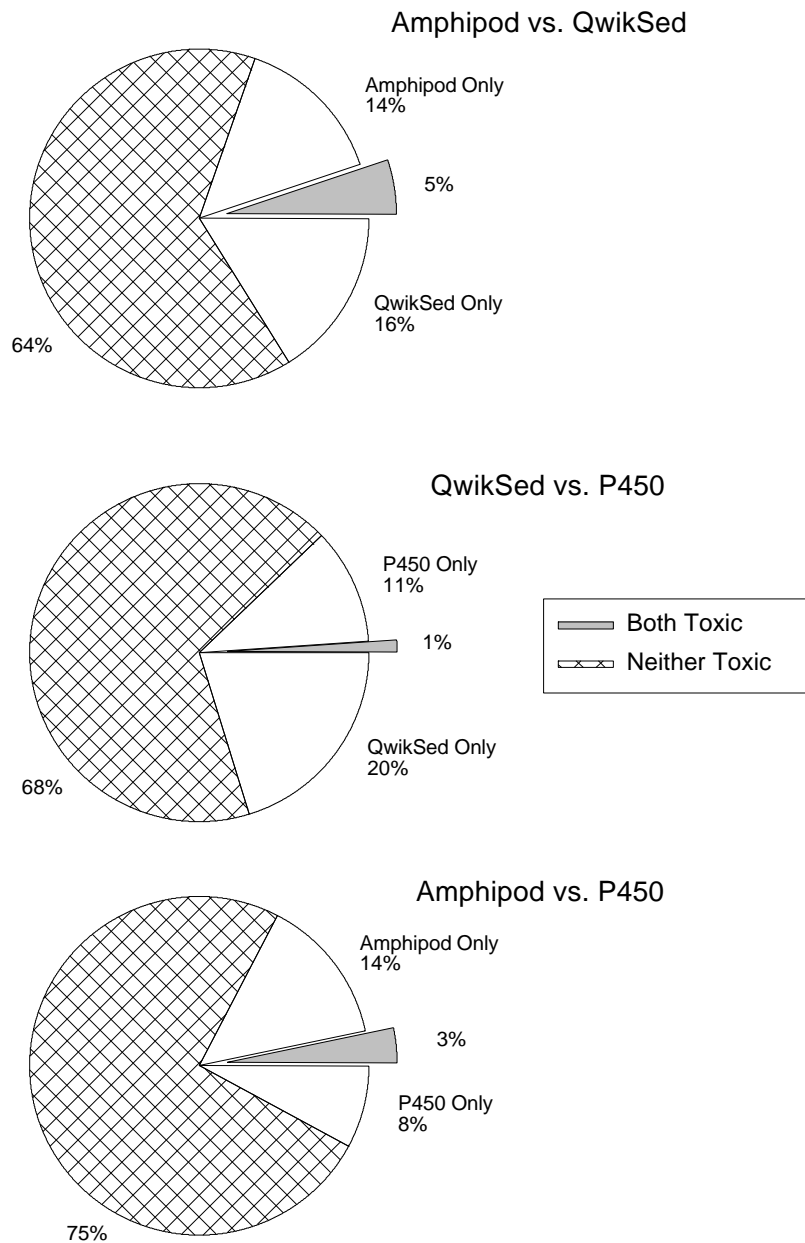
Pairwise comparisons of toxicity classifications based upon the amphipod, QwikSed, and HRGS test results indicated that the tests were in agreement for most of the samples (Figure V-1). The amphipod survival and HRGS induction tests had the highest percentage of agreement, with 78% of samples classified as nontoxic or toxic by both methods. Classifications based upon the QwikSed test had a similar level of agreement (69%) with either the amphipod or HRGS test results.

Most of the agreement among tests occurred in the designation of samples as nontoxic. Relatively few samples were classified as toxic by any two test methods. Approximately 18-26% of the samples identified as toxic based upon amphipod survival were also classified as toxic by the HRGS or QwikSed tests, respectively (Figure V-2). Agreement on toxicity classification was lower between HRGS and QwikSed (8%).

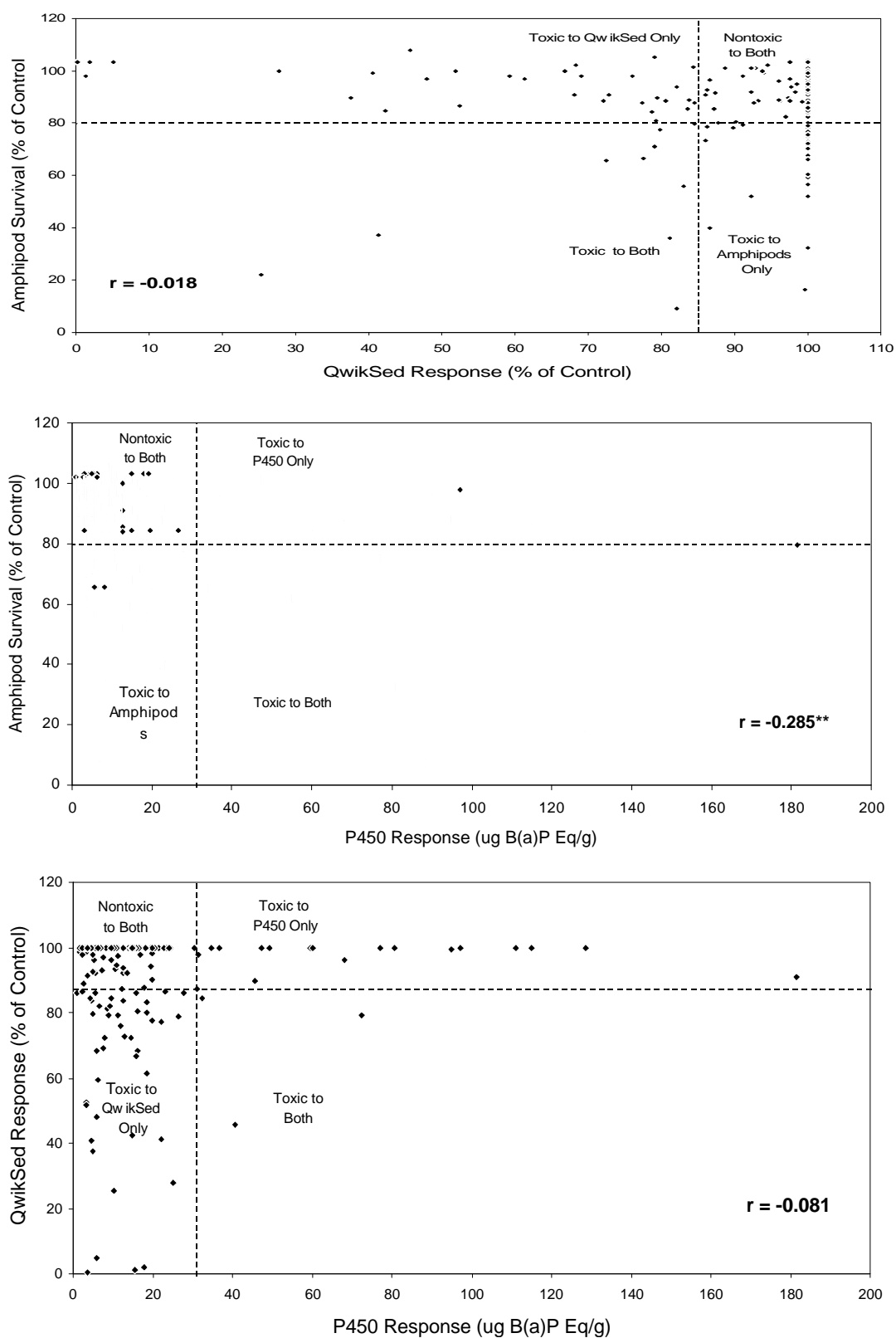
When toxicity was present in a sample, the magnitude of response was variable among the three test methods (Figure V-2). Relatively few samples produced a strong toxic response in any two tests. No samples were identified as toxic by all three methods. Fifty-six percent of the samples were identified as nontoxic by all three methods, however.

A weak significant correlation was found between the magnitude of response for amphipod survival and HRGS induction (Figure V-2). This correlation had a negative sign, indicating the expected trend of reduced amphipod survival in sediments containing elevated contaminant concentrations. No correlation was present between QwikSed and either amphipod or HRGS results.

Stronger correlations were present between sediment total organic carbon (TOC) or grain size (percent fines) and toxicity (Appendix F). Amphipod survival was significantly correlated with TOC ( $r = -0.49$ ) and grain size ( $r = -0.56$ ). Although survival tended to be lower in some organically enriched or fine sediments, many of the samples containing the highest TOC concentrations or the finest sediments were classified as nontoxic. The HRGS test responses were also found to be significantly correlated with increased TOC ( $r = 0.70$ ) or increased sediment fines ( $r = 0.53$ ). The QwikSed test was not significantly correlated with either sediment parameter.



**FIGURE V-1. Results of toxicity classifications for each pair of toxicity tests. Each pie diagram shows the percentage of stations for which both tests yield either the same result (either toxic or not toxic) or disagree (only one test detected toxicity).**



**FIGURE V-2. Comparison of indicator responses for sediment samples. Dashed reference lines indicate toxicity classification thresholds. The P450 plots do not include one high sample (903  $\mu\text{g/g}$ ). Asterisks indicate a significant correlation.**

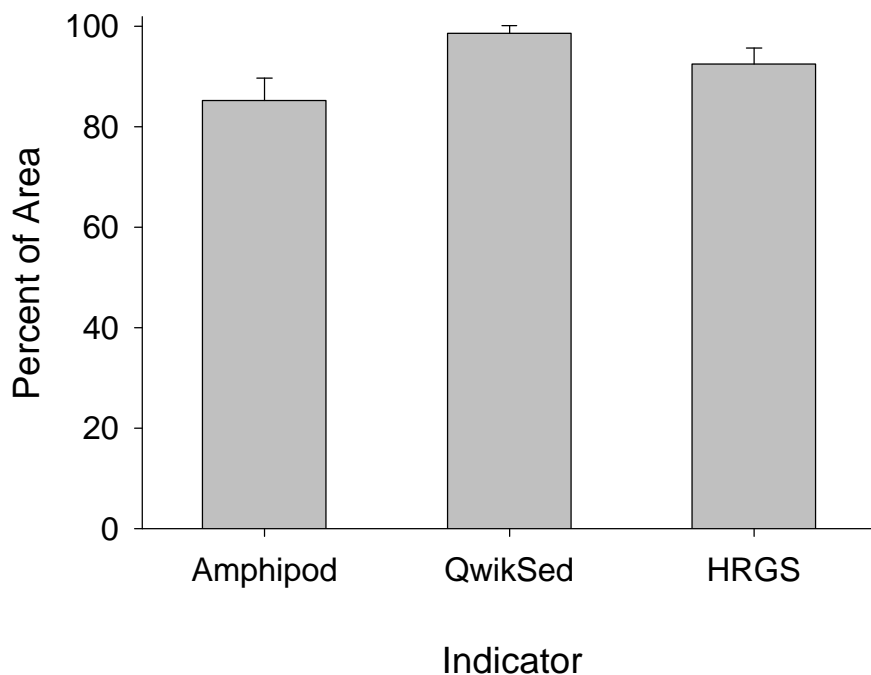


## VI. REGIONAL ASSESSMENT OF TOXICITY

Sediments from most areas of the SCB were of good quality. Sediments were nontoxic in 85-99% of the area studied, depending upon the indicator used (Figure VI-1). The amphipod test detected the largest area of toxicity, 504 km<sup>2</sup> (Table VI-1). The QwikSed test identified the smallest area of toxicity, 48 km<sup>2</sup>.

Each of the indicators were consistent in detecting the smallest extent of toxicity in the shallow offshore stations. The amphipod and HRGS tests each identified 3% of the shallow area as toxic, which represented a single station, while no toxicity was found using the QwikSed test. Estimates of the spatial extent of toxicity varied among indicators for the other strata, with no single indicator being most sensitive for all strata (Figure VI-2).

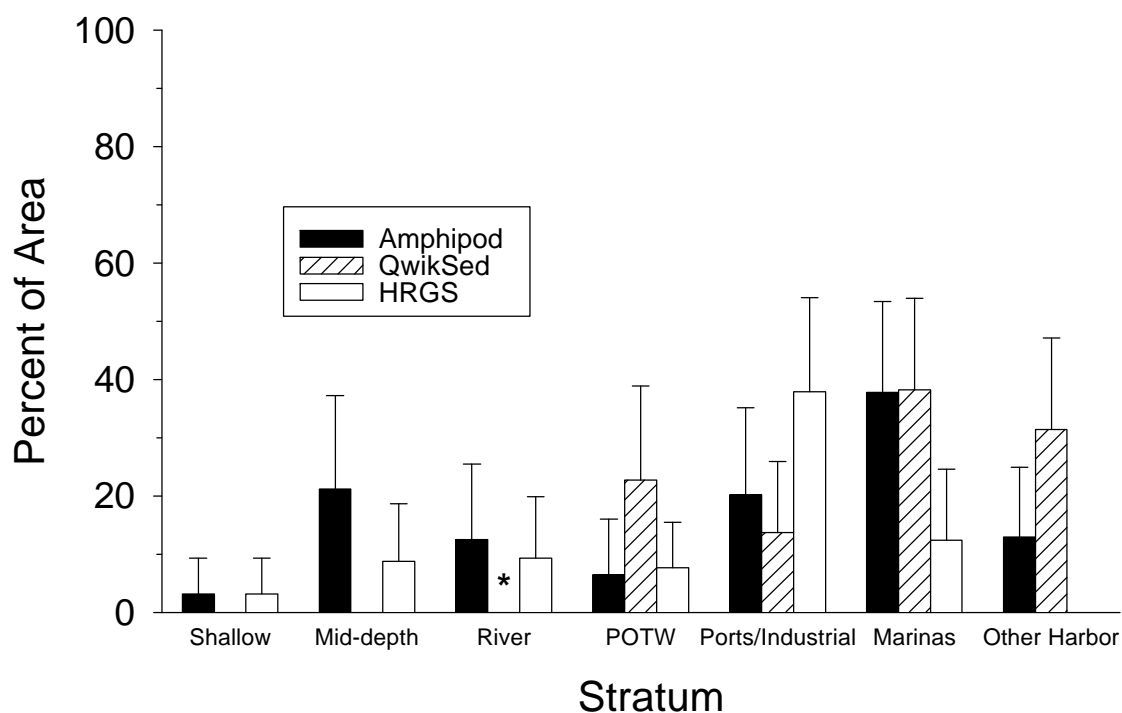
Strata located within bays and harbors contained the largest relative area of toxicity compared to offshore strata for each indicator. The amphipod and HRGS tests detected the highest percentage of toxic area within the port/industrial (20 and 38%, respectively) and marina strata (38 and 12%, respectively), while the QwikSed test identified the largest toxic area within the marina (38%) and other category strata (31%). The tests identified toxicity in 6-23% of POTW outfall areas and in 9-12% of the areas near river mouths. Areal estimates of toxicity within the mid-depth stratum varied markedly between indicators, from 0% (QwikSed) to 21% (amphipod). The incidence of amphipod toxicity in mid-depth areas represented 390 km<sup>2</sup>, accounting for most of the difference in bight-wide estimates of toxic area relative to QwikSed and HRGS.



**FIGURE VI-1. Percent of Southern California Bight not toxic to each indicator.**

**TABLE VI-1. Percent of area in each stratum classified as toxic by indicator type.**

Stratum	Area Km <sup>2</sup>	Amphipod		QwikSed		HRGS	
		Moderate	High	Moderate	High	Potential	High
Shallow	1,066	3	0	0	0	0	3
Mid-depth	1,838	21	0	0	0	3	6
River Mouths	165	10	3	na	na	6	3
POTW Outfall							
Areas	186	6	0	5	18	8	0
Ports/Industrial	27	18	2	15	0	15	23
Marinas	17	21	17	31	7	8	4
Other	108	13	0	22	10	0	0
SCB	3,407	14.3	0.1	0.8	0.6	2.6	4.9



**FIGURE VI-2. Percent of area (+ 95% confidence interval) found to be toxic in each stratum. \*Only one sample from river stratum analyzed for QwikSed.**

The results for each of the three toxicity indicators were combined to provide an integrated assessment of sediment quality. The results were weighted relative to the severity of response and perceived ecological relevance of the indicator. The greatest weight was given to the amphipod survival results because the amphipod test used test conditions that were most similar to the habitat of interest (benthic species, direct sediment exposure) and a response with high biological significance (survival). Lesser weight was assigned to the QwikSed and HRGS test results because these methods used sublethal responses to a test matrix that had been manipulated by elutriation or extraction procedures. The weighted results were combined to classify each station into one of three levels of concern (Table VI-2).

The high concern category included stations where reduced survival of some benthic animals was expected. All stations that were highly toxic to amphipods (<50% survival) were classified as areas of high concern. Stations were also placed into this category if they produced moderate amphipod toxicity and were also identified as toxic in either the QwikSed or HRGS test. If the QwikSed and P450 tests were classified as highly toxic, a station was defined as an area of high concern even though the amphipod test showed no toxicity.

Areas of potential concern included stations where a less severe (sublethal) toxic response was measured or the results were inconsistent between the survival and sublethal tests. Stations in this category represented areas where marginal effects may be present, resulting in greater uncertainty about the significance of impaired sediment quality.

This category included stations that were usually not toxic to amphipods but produced some evidence of toxicity in at least one of the other two bioassays. Areas of no concern demonstrated no amphipod toxicity and either no toxicity or inconsistent evidence of a moderate toxic response in the other two tests.

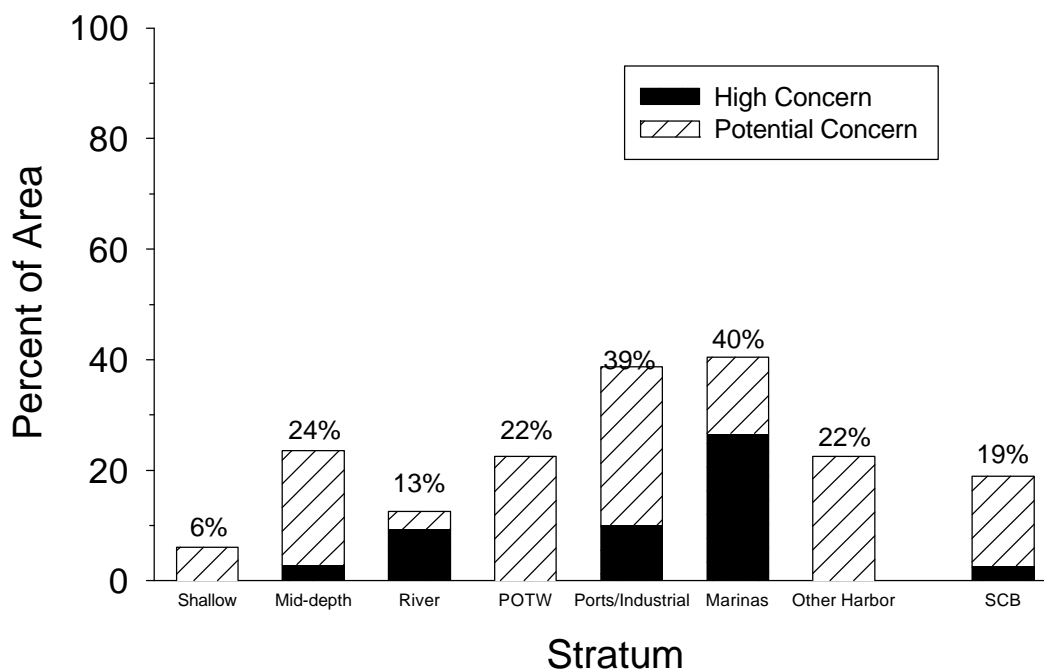
The integrated classification strategy was applied to the 241 stations that were assessed by both the amphipod and HRGS tests. QwikSed test results were not available for 68 of these stations; a nontoxic QwikSed response was assigned to them for the purpose of applying the classification strategy.

Areas of potential or high concern were present in each of the strata and comprised 19% of the area (644 km<sup>2</sup>) of the SCB (Figure VI-3). Most of the affected area was classified in the potential concern category; only 2.7% of the SCB (92 km<sup>2</sup>) was classified in the high concern category. Among strata, port/industrial and marina areas had the largest total percent of area in the two categories of concern, 39 and 40%, respectively. Marinas contained the largest relative area and most severe effect, with 27% of the area classified in the high concern category. Areas of high concern were also present in 10% of port/industrial areas and 9% of river areas.

The spatial extent of toxicity was intermediate among the mid-depth, POTW, and the other bays and harbors area strata (22-24% of area). Shallow areas were least affected, with only 6% of the area classified in the potential concern category.

**TABLE VI-2. Strategy for determining levels of concern from results of three types of sediment toxicity tests.**

QwikSed Toxicity	HRGS Toxicity	Amphipod Toxicity		
		High	Moderate	Nontoxic
<b>High</b>	<b>High</b>	High Concern	High Concern	High Concern
	<b>Potential</b>	High Concern	High Concern	Potential Concern
	<b>Nontoxic</b>	High Concern	High Concern	Potential Concern
<b>Moderate</b>	<b>High</b>	High Concern	High Concern	Potential Concern
	<b>Potential</b>	High Concern	High Concern	Potential Concern
	<b>Nontoxic</b>	High Concern	High Concern	No Concern
<b>Nontoxic</b>	<b>High</b>	High Concern	High Concern	Potential Concern
	<b>Potential</b>	High Concern	High Concern	No Concern
	<b>Nontoxic</b>	High Concern	Potential Concern	No Concern



**FIGURE VI-3. Percent of area of concern based upon joint toxicity classification.**

## VII. DISCUSSION

Sediment toxicity evaluations were successfully conducted using the amphipod, QwikSed, and HRGS tests. These tests met the performance criteria specified in the Bight'98 Quality Assurance Plan for 98% of the samples. An interlaboratory comparison demonstrated that the amphipod toxicity results were comparable among laboratories.

The successful application of three toxicity indicators during Bight'98 has enabled two of the objectives of the study to be attained: (1) estimation of the area of toxic sediments among various strata of the SCB and (2) comparison of the responses of different toxicity test methods. The final objective, evaluation of relationships between toxicity and other indicators of sediment quality, cannot be accomplished at this time because the analysis of chemistry and benthic biology samples is still in progress. The results of this evaluation will be presented in a separate report that integrates the results of all indicators.

The toxicity results indicate that the majority (81%) of the SCB had good sediment quality in 1998. Toxicity was generally most severe (greatest test response) and most prevalent (largest percent of area affected) in developed areas of bays and harbors (port/industrial and marina strata), a result that is consistent with previous studies in southern California (Anderson *et al.* 1988, Fairey *et al.* 1998), and throughout the nation (Long 2000, *in press*). Greater toxicity is to be expected in bays and harbors because these locations receive contaminant inputs from many sources (e.g., urban runoff, industrial spills, boating activity), which adsorb onto the fine-grained bottom sediments. Most bays and harbors are depositional environments, which facilitate the retention and accumulation of contaminated sediments over time.

The presence of toxicity was unambiguous in 3% of the SCB; these were identified as areas of high concern in the integrated classification strategy because high amphipod mortality was measured or multiple indicators detected toxicity. These areas were located primarily in marinas, port/industrial areas, and near the Los Angeles River. Variable or weaker toxicity results were obtained for a larger number of stations (classified as areas of potential concern), representing 16% of the SCB and distributed among all of the strata. These areas would be classified as toxic if the data were interpreted according to the criteria used in NOAA or EMAP studies. The classification of these stations as areas of potential concern in this report reflects a lower level of confidence in the ecological significance of the data, which is prompted by the lack of corroborating data describing contaminant concentrations and benthic community composition at these stations. These data are needed to provide the weight of evidence necessary to determine whether the toxicity results are ecologically significant or associated with anthropogenic activities.

The identification of 379 km<sup>2</sup> of mid-depth areas in the potential concern category, primarily based upon toxicity to amphipods, is in contrast to the 1994 SCBPP results that showed no acute toxicity in the same region (Bay *et al.* 1998). A different amphipod test species (*Ampelisca abdita*) was in the 1994 and differential sensitivity between these species may have been responsible for some of the difference in results between studies. Recent studies in California indicate that tests with *E. estuarius* are more likely to detect sediment toxicity than tests with *A.*

*abdita* (B. Anderson, pers. Comm.). Evidence of interstitial water toxicity in this area was observed during the SCBPP, however. Most of these toxic Bight'98 mid-depth stations were located in Santa Barbara and Ventura counties, where large point sources of contaminants are absent. Toxicity at these stations may be related to the presence of numerous natural oil "seeps," or flows, in the area. The highest P450 HRGS response to sediments (903  $\mu\text{g B[a]PEq/g}$ ) was measured for a station located off of Coal Oil Point, a known seep area. Forthcoming sediment chemical analysis results may help to reveal the influence of natural hydrocarbons on SCB sediment toxicity.

The Bight'98 amphipod toxicity results for bays and harbors (13-38% of the area, depending upon the stratum) fell within the range reported in previous studies by NOAA and the State Water Resources Control Board. Studies conducted between 1992 and 1994 reported toxicity in 66% of San Diego Bay (Fairey *et al.* 1998), 14% of Los Angeles/Long Beach Harbor (Long 2000, *in press*), and 58% of other small bays and marinas (Anderson *et al.* 1997). Amphipod toxicity throughout the SCB (14.8% of the area) was higher than the national average for estuarine areas of 5.9% reported by Long (2000).

A pronounced temporal difference in the extent of amphipod toxicity for San Diego Bay was observed. Nine percent of San Diego Bay was toxic to *E. estuarius* in 1998, while tests with a different species, *Rhepoxynius abronius*, detected toxicity in 66% of the bay (Fairey *et al.* 1998).

The use of different amphipod test species is a potential cause of the temporal differences in toxicity results for San Diego Bay and the SCB. Most of the earlier tests were conducted with either *Rhepoxynius abronius* or *Ampelisca abdita*. The relative sensitivity of *E. estuarius* and these species has been studied to a limited extent. Sensitivity to single contaminants varies between species in an unpredictable manner. For example, *E. estuarius* is less sensitive than *R. abronius* to fluoranthene and cadmium (DeWitt *et al.* 1989), yet is slightly more sensitive to DDT. All three species have a similar sensitivity to crude oil (Weston 1996). The sensitivity of *E. estuarius* to contaminated field sediments has been found to be similar to other amphipods (DeWitt *et al.* 1989, Schlekot *et al.* 1995). Analyses of sediment toxicity data from many field studies indicate that *R. abronius* and *A. abdita* respond similarly to sediments having comparable contaminant concentrations (Field *et al.* 1999). Thus, differences in contaminant sensitivity between species may not be a significant factor in field studies, perhaps because the joint toxicity of multiple contaminants obscures species-specific differences. Concurrent tests of southern California sediments using multiple amphipod species are needed to determine whether the choice of amphipod species has a significant influence on toxicity estimates for the SCB.

The HRGS test has been applied previously to bay and harbor sediments in southern California and throughout the nation. The Bight'98 results indicate a greater prevalence of samples with potential or high toxicity (7.2%) compared to studies of southern California coastal bays (0%) or studies throughout the nation (0-3.4%) as reported by Anderson *et al.* (1999) and Long (2000, *in press*). The QwikSed test is a relatively new method that has not been applied in other regional surveys.

A broad level of agreement was found among the three test methods (amphipod survival, QwikSed, and HRGS). All of these tests were in agreement for most of the samples classified as nontoxic and each test also identified the greatest toxicity in bay and harbor areas. However, the test methods responded differently to most of the sediments where toxicity was present. This conclusion is supported by the lack of strong correlations between the test results and the low percentage of samples that were identified as toxic by more than one indicator. Such differences are often observed in regional sediment toxicity studies, where samples with diverse physical and chemical characteristics are evaluated (Long *et al.* 1998).

Differences in sediment toxicity test responses are to be expected, considering that each of the test methods is likely to vary in sensitivity to specific contaminants. For example, the HRGS test is sensitive to a select group of biologically active organics (dioxins, furans, PAHs, and PCBs), but not to metals or pesticides. Sublethal tests using phytoplankton or invertebrate larvae are often more sensitive to short-term chemical exposure than are adult crustaceans (Lapota *et al.* 1999). Such differences in test responses among indicators is desirable in sediment quality surveys as it indicates that each method is contributing new information about the potential toxicity of the sample. The use of only one toxicity test method cannot provide a complete measurement of the toxicity of a sample to the diversity of benthic organisms inhabiting the SCB.

Some of the differences in toxicity test responses may also be due to undesired artifacts or interferences related to the laboratory test procedure. Sample preparation steps required by each test method, such as sediment homogenization, elutriate preparation, or solvent extraction, can alter the partitioning and/or bioavailability of sediment contaminants and can lead to different responses among tests. The relative role of artifactual versus biological variations in producing the differences in Bight'98 test responses is difficult to determine at present, as corresponding sediment chemistry and benthic community data have not yet been analyzed.

The diversity of responses obtained with multiple indicators presents a challenge for those tasked with making an assessment of sediment quality, who must find a way to reconcile conflicting responses from multiple indicators. The Bight'98 toxicity study used a relatively conservative strategy that weighted the results based upon assumptions about the ecological significance of each indicator's response. The efficacy of this strategy for predicting impaired sediment quality is not known at present, but will be evaluated through comparisons with benthic community responses and sediment chemistry in subsequent Bight'98 regional monitoring program reports. Until these comparisons have been made, our assessments of the categories of concern should be viewed as preliminary.

## VIII. CONCLUSIONS

The Bight'98 sediment toxicity study produced the most comprehensive regional assessment of sediment quality in the SCB. This study marks the first time that offshore and bay/harbor sediment quality has been assessed at the same time, using the same methods. Two recently developed test methods, QwikSed and P450 HRGS, were used for the first time in many locations. The first interlaboratory comparison of sediment toxicity test results among southern California laboratories was also conducted as part of Bight'98. Analysis of the results by the Toxicology Committee, representing the participating laboratories and other survey partners, has resulted in the following conclusions:

- **Sediment toxicity was evident in 19% of the sediments of the SCB study area.**

Sediment samples from stations representing 644 km<sup>2</sup> produced toxicity in at least one of the tests. The majority (552 km<sup>2</sup>) of this area was classified in the potential concern category, representing areas where toxicity was less severe or inconsistently detected among the indicators. Toxicity was more severe in sediments representing 2.7% of the SCB (92 km<sup>2</sup>) that were classified as areas of high concern; these sediments were almost exclusively located within harbors and bays.

- **Sediments located within bays and harbors or near river mouths contained the greatest severity of toxicity.**

While evidence of acute or sublethal toxicity was detected in every stratum sampled, each indicator detected the strongest responses or the greatest extent of toxicity within bays and harbors. The strongest toxic responses in the amphipod test (<50% survival) were produced by sediments from marina, port/industrial, and river mouth areas. QwikSed toxicity was highest in POTW outfall, marina, and other bay/harbor areas. Strong HRGS responses (>60 µg/g Benzo[a]pyrene equivalents, a level associated with degraded infauna) were most frequently produced by sediments from port/industrial areas, although similar responses were produced by a few stations in marina, river mouth, mid-depth, and shallow areas. Each test method identified shallow areas as having the lowest percentage of toxic area.

- **Different patterns of response were obtained for each toxicity indicator.**

While each of the tests produced a similar toxicity classification for most of the stations, different response patterns were evident. Correlation analysis indicated only a weak relationship between the amphipod and HRGS results for the same stations, and no relationship was found between QwikSed and the other tests. A relatively low rate of agreement (8-26%) was observed between pairs of tests in classifying a station as toxic; and no station was classified as toxic by all three tests. These results indicate that each test method responded to different characteristics of the sediment and provided new information about the sediment samples.



- **Amphipod toxicity test data were of high quality and comparable among all of the participating laboratories.**

Each of the participating laboratories produced results that met test acceptability criteria. An interlaboratory comparison demonstrated that the results from all seven participating laboratories were usually not significantly different from one another. The variability in reference toxicant results among the laboratories was the same as the within-laboratory variability. This result indicates the use of multiple laboratories did not result in increased measurement error during the study.

## IX. RECOMMENDATIONS

- **Determine the relationship between indicator threshold exceedence and benthic community effects.**

The choice of toxicity classification thresholds is usually based upon statistical considerations (e.g., significantly different from control) or arbitrary numeric values (e.g., 50% response). Such thresholds provide an important element of standardization to toxicity assessments, but their correspondence to biological impact is poorly understood. Acute toxicity test responses are generally assumed to have high ecological relevance, but the significance of a 20% change in organism growth or metabolism during a toxicity test is more difficult to determine. Once the Bight'98 benthic ecology data are available, analyses should be conducted to determine the relationship between toxicity test response and benthic effects. The results of these analyses should be used to evaluate (and improve) the response thresholds and weighting used to produce the toxicity assessments presented in this report. Understanding the predictive ability of sediment toxicity will be beneficial to the planning of future monitoring activities.

- **Identify the cause of toxicity in bays/harbors and mid-depth areas.**

The greatest spatial extent and severity of toxicity was present in bays and harbors, confirming the results of earlier toxicity surveys. The persistent nature of sediment toxicity in areas such as Newport Bay and San Pedro Bay indicates that sediment quality is not improving. Sediment chemistry data should be analyzed and additional toxicity identification studies conducted to determine the cause of toxicity in these areas so that potential sources can be identified and load reduction/site cleanup activities effectively planned. Follow-up toxicity studies should also be conducted in the mid-depth area of Santa Barbara and Ventura counties. The presence of acute sediment toxicity in this area was not expected due to the absence of large point source discharges and may be due to natural factors (e.g., oil seeps). Confirmation of the toxicity results for this area may help elucidate some of the non-anthropogenic factors causing variability in test response, thus facilitating improved application and interpretation of toxicity tests in other programs.

- **Investigate causes of non-concordance between different sediment toxicity tests.**

The variability in results between the different Bight'98 sediment toxicity test methods creates uncertainty in the interpretation of the data. The cause of this variability is unknown, although it is probably the result of several factors, such as species-specific differences in contaminant sensitivity and differences in contaminant exposure related to test sample handling. The efficacy of these tests for predicting impaired sediment quality needs to be evaluated so the results can be used appropriately in environmental management decisions. An essential first step is to compare the toxicity results to the sediment contamination and benthic community data among samples. Following this step, field and laboratory experiments should be conducted to evaluate issues such as contaminant sensitivity, test reproducibility, and the influence of non-contaminant sediment factors.

- **Conduct periodic regional surveys to assess temporal trends.**

The Bight'98 sediment toxicity study identified a larger amount of toxicity in offshore strata and less toxicity in San Diego Bay compared to prior studies. The cause of these trends has not yet been determined, but changes in sediment quality, natural variability, and differences in test methods may all play a role. A long-term database of sediment toxicity data using comparable methodology is needed in order to differentiate between changes in sediment quality and natural/measurement variability. Distinguishing between natural and anthropogenic effects is needed to select appropriate management actions in response to the detection of toxicity. The establishment of a long-term sediment toxicity database will also permit the evaluation of the correspondence between sediment toxicity, which measures present conditions, and benthic community effects, which integrate effects over longer timescales. Future regional surveys should continue to compare the performance of rapid and inexpensive assays, such as HRGS and QwikSed. These methods provide cost-effective alternatives to conventional tests of contamination and toxicity and could facilitate the collection of more spatially or temporally extensive data. But additional study of these alternative methods is needed in order to evaluate their relevance to ecological impacts.

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**APPENDIX A. PARTICIPANTS IN THE SOUTHERN CALIFORNIA BIGHT  
1998 REGIONAL MONITORING PROGRAM (BIGHT '98). <sup>a</sup> Denotes  
participants in the sediment toxicity component.**

AES Corporation  
Algalita Marine Research Foundation  
Aliso Water Management Authority (AWMA)  
Aquatic Bioassay and Consulting Laboratories, Inc.  
California Coastal Conservancy  
Central Coast Regional Water Quality Control Board  
Channel Islands National Marine Sanctuary (CINMS)  
Chevron USA Products Company  
Cities and County of Riverside Stormwater Program  
City of Long Beach  
City of Los Angeles Environmental Monitoring Division (CLAEMD)<sup>a</sup>  
City of Los Angeles Stormwater Division  
City of Oceanside  
City of Oxnard  
City of San Diego<sup>a</sup>  
City of Santa Barbara  
City of Ventura  
Columbia Analytical Services<sup>a</sup>  
Commission for Environmental Cooperation  
Divers Involved Voluntarily in Environmental Rehabilitation & Safety (DIVERS)  
Encina Wastewater Authority  
Goleta Sanitation District  
Houston Industries, Inc.  
Instituto de Investigaciones Oceanologicas, Universidad Autonoma de Baja California  
(UABC)  
Los Angeles Department of Water and Power  
Los Angeles County Department of Beaches & Harbors  
Los Angeles County Department of Health Services  
Los Angeles Regional Water Quality Control Board<sup>a</sup>  
Los Angeles County Sanitation Districts (LACSD)  
Marine Environmental Consultants (MEC)<sup>a</sup>  
Marine Corps Base Camp Pendleton  
National Fisheries Institute of Mexico (SEMARNAP)  
NOAA-NOS International Programs Office  
NRG Energy, Inc.  
Orange County Environmental Health Division  
Orange County Public Facilities and Resources Department (OCPFRD)  
Orange County Public Health Laboratory  
Orange County Sanitation District (OCS)<sup>a</sup>  
San Bernadino County Stormwater Program  
San Diego County Dept. of Environmental Health

**APPENDIX A (continued). Participants in the Southern California Bight 1998 Regional Monitoring Program (Bight '98). <sup>a</sup> Denotes participants in the sediment toxicity component.**

San Diego Interagency Water Quality Panel (Bay Panel)  
San Diego Regional Water Quality Control Board  
San Elijo Joint Powers Authority  
Santa Ana Regional Water Quality Control Board  
Santa Barbara Public Health Department  
Santa Monica Bay Restoration Project  
Southeast Regional Reclamation Authority (SERRA)  
Southern California Coastal Water Research Project (SCCWRP)<sup>a</sup>  
Southern California Edison (SCE)  
Southern California Marine Institute (SCMI)  
State Water Resources Control Board (SWRCB)<sup>a</sup>  
Surfrider Foundation  
USC Wrigley Institute for Environmental Studies (WIES)  
University of California, Santa Barbara  
University of California, Davis, Marine Pollution Studies Lab<sup>a</sup>  
US EPA Region IX  
US EPA Office of Research and Development  
US Geological Survey  
US Navy, Space & Naval Warfare Systems Center, San Diego (USN)<sup>a</sup>  
Ventura County Health Department

## **APPENDIX B. INTERLABORATORY COMPARISON OF SEDIMENT TOXICITY TESTS WITH THE AMPHIPOD *EOHAUSTORIUS ESTUARIUS***

### **Abstract**

The sediment toxicity assessment program of the Southern California Bight 1998 Regional Monitoring Survey (Bight '98) was a coordinated effort with seven laboratories sharing the responsibility for conducting 10-d amphipod tests (*Eohaustorius estuarius*). An interlaboratory comparison exercise was conducted prior to testing Bight '98 field samples. In order to assess laboratory performance with samples collected in the field, sediments from four stations in Los Angeles/Long Beach Harbor were tested along with the animal collection site control. All laboratories successfully performed the sediment test and associated reference toxicant test. Statistically significant differences in amphipod mean survival were found among some laboratories for the field-collected sediments. However, precision of the results did not appear to be reduced by having multiple laboratories participate in the testing. The laboratories demonstrated excellent concordance (Kendall's  $W = 0.91$ ) in ranking the field-collected sediments by toxicity. Agreement on classifying the sediments into the Bight '98 toxicity categories (not toxic, moderately toxic, highly toxic) was good for sediments with mean survival values that differed sufficiently from the threshold.

### **Introduction**

Regional surveys such as Bight '98 require good QA/QC programs to ensure quality results. The number of potential variables in the results increases with the number of participating organizations. A good QA/QC program ensures that the sharing of responsibilities is a positive feature of the program and does not compromise the quality of the data. As part of the Bight '98 QA/QC plan, an interlaboratory comparison exercise was conducted in order to assess competence and comparability among the laboratories prior to the start of the Bight '98 sampling and testing program. Although a majority of the laboratories had experience with performing 10-d amphipod tests, most had not yet worked with *Eohaustorius*. One laboratory, however, had extensive experience with *Eohaustorius* and contributed valuable knowledge to the group.

The Bight '98 planning process necessitated an accelerated preparation schedule for the laboratories. The committee recommended that each laboratory complete the interlaboratory comparison and two additional preliminary tests before beginning the Bight '98 testing. Thus, the exercise had to fulfill dual roles: (1) to allow the laboratories to gain experience working with *Eohaustorius* and, at the same time, (2) to provide comparison data for QA assessment. Since Bight '98 toxicity samples were not split among laboratories, the interlaboratory exercise provided the only opportunity to



compare test results from different laboratories using split samples of field-collected sediments.

The nature of toxicity testing presents some challenges to an interlaboratory comparison. Toxicity is not an indicator that is measured directly; rather it is assessed based upon a biological response. Interlaboratory agreement for some environmental indicators (i.e., directly measured chemical parameters) can be assessed by distributing a coded sample of a standard reference material with a known value (Burton *et al.* 1996). The results are assessed based upon how close the laboratories are to the known value (accuracy) as well as by examining interlaboratory variability in the answers (precision). However, the choice of measures for the assessment of interlaboratory agreement in toxicity testing is somewhat more limited. While clean reference sediments are frequently included in bioassays as a negative control (expected to have no toxic response), reference sediments having “known” toxicity values are not available.

It is important to assess the ability of the participating laboratories to detect responses in contaminated sediments (Schlekat *et al.* 1995). Thus, in order to make the most valuable interlaboratory comparison, it is important to include field sediments with a range of expected toxicity responses. The sediments are compared on a relative basis instead of an absolute one. The closest approach to a “true” value is a consensus value based upon the results of the group. Therefore, results from testing field sediments in multiple toxicology laboratories are judged on the precision of the results rather than on accuracy.

This paper presents the results of the interlaboratory comparison exercise conducted prior to the start of Bight '98 sampling. The first objective of the study was to assess whether each laboratory was able to perform the 10-d *Eohaustorius* test in an acceptable manner. This criterion was measured using two methods: (1) by evaluating attainment of test acceptability criteria and (2) by comparing relative reference toxicant performance. The second objective was to assess the degree of agreement in the test responses observed by the laboratories. This objective was accomplished by comparing the toxicity results of the field-collected sediments.

## **Methods**

### **Experimental Design**

The design of the exercise was as follows: four field-collected sediments were collected and homogenized by one agency and then divided among seven laboratories for toxicity testing. Each laboratory also concurrently tested sediment from the animal collection site (negative control) and a specified cadmium reference toxicant series. Several important steps were taken in order to control experimental variables outside of the individual laboratories: (1) test sediments and reference toxicant stocks were simultaneously distributed from one central source using identical containers. (2) test organisms were obtained from a common commercial supplier out of one collection batch

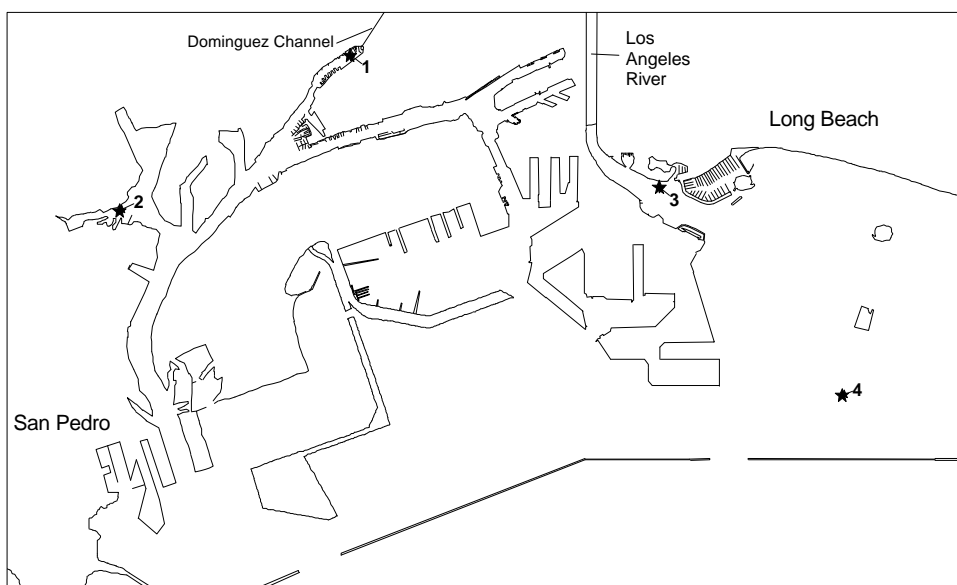
and distributed to each of the laboratories, and (3) each laboratory began the bioassay on the same day.

### **Sample Collection and Handling**

Sediment sampling was conducted on May 7, 1998. Sediment samples were collected from four stations in Los Angeles/Long Beach Harbor: LAH1 (33° 46.564', 118° 14.608'), LAH2 (33° 45.343', 118° 16.787'), LAH3 (33° 45.529', 118° 11.684'), and LAH4 (33° 43.890' 118° 9.955') (Figure B1). These sites were chosen by screening existing amphipod toxicity data (SWRCB 1996) for stations with a wide range in toxicity response. Sediment samples were collected with a 0.1 m<sup>2</sup> modified van Veen grab. At a given station, multiple grab samples were taken to provide 12 L of sediment. A plastic (high-density polyethylene [HDPE]) scoop was used to collect sediment from the top 4 cm of the undisturbed surface material in the grab. Contact with sediment within 1 cm of the side of the grab was avoided in order to minimize contamination. The sediment was transported back to the laboratory in polycarbonate containers on ice. Once back at the laboratory, the sediment was homogenized in a polyethylene bucket using an overhead mixer. The homogenized sediment was transferred to 950 mL and 250 mL polyethylene containers.

The laboratories participating in the exercise were: Aquatic Bioassay and Consulting, Marine Pollution Studies Laboratory, City of Los Angeles Environmental Monitoring Division, City of San Diego Ocean Monitoring Program, MEC Analytical Systems, County Sanitation Districts of Orange County, and SCCWRP. Each laboratory received 1.2 L of sediment from each of the four stations. The sediment was shipped overnight to each laboratory or picked up directly by the laboratory.

Test organisms, *Eohaustorius estuarius*, were obtained from Northwest Aquatic Sciences (collection site: Beaver Creek, Oregon). The animals were collected on May 6, 1998, and delivered to each laboratory by Federal Express on May 7, 1998. The overlying water in the sediment was approximately 20 ppt. These containers were emptied into larger tubs at each laboratory and 20 ppt water and aeration were added. The amphipods were held in constant illumination at 15° C until the initiation of the test.



**FIGURE B1. Locations of sites sampled in Los Angeles/Long Beach Harbor for interlaboratory comparison.**

### **Test Procedures**

Sediment toxicity was determined using a 10-d amphipod survival test (EPA/600/R-94/025). Sediment toxicity tests were conducted in 1 L glass test containers. Sediment was added to the test containers 1 d prior to the start of the test (May 11). Sediment samples were thoroughly mixed and then added to the test containers to form a sediment layer approximately 2 cm deep. Filtered seawater (20 ppt) was slowly added until a final volume of 800 mL was reached. Pipettes connected to an air source provided aeration. Test containers were then allowed to equilibrate overnight. Each sample consisted of five randomly arranged replicates, along with an extra container to provide samples for water quality. For each batch of samples tested, a negative control (consisting of test animal collection site sediment) was included.

At the start of the test (May 12, 1998), amphipods were added randomly until a total of 20 animals per container was present. Tests were conducted at 15° C under constant illumination. Test animals were exposed to the sediment samples for 10 d. Test containers were checked daily for air and for any dead animals or animals stuck to the surface of the water. Any floating animals were submerged by gently pushing them beneath the surface with a probe. At the end of the exposure period (May 22, 1998) the sediment was screened through a 0.5 mm screen and the number of surviving amphipods was recorded.

Concurrently with the sediment toxicity test, a cadmium reference toxicity test was conducted. A standard cadmium stock solution (1,000 mg/L) was prepared and distributed to the participating laboratories. The aqueous phase reference toxicant test consisted of three replicates of 5 dilutions, plus control. The dilutions were 0.32, 1.00, 3.20, 5.60, and 10.00 mg/L. A sample of the 10.00 mg/L concentration was analyzed for verification purposes. At the beginning of the test (May 12, 1998), 10 amphipods per replicate were added randomly to each test container and exposed to the reference toxicant for 4 d. At the end of 4 d (May 16, 1998) the number of surviving animals was recorded and the LC<sub>50</sub> (median lethal concentration) was calculated.

Initial water quality (temperature, pH, dissolved oxygen, salinity, and total ammonia) was recorded from the overlying water from the extra test container. Interstitial water quality was also obtained from sediment collected from the same container centrifuged at 3,000 g for 20 minutes. Temperature, pH, salinity, and dissolved oxygen of the overlying water were also recorded at the end of the exposure period. All water quality measurements were measured with laboratory-approved equipment and procedures. Water quality measurements for the reference toxicant test were similar to the sediment phase of the test.

## **Data Analysis**

Absolute agreement was assessed by comparing mean survival among the laboratories. The mean percent of survival rate was calculated for each sediment sample (grouped by laboratory). The data were then normalized by dividing the sample mean by the appropriate control mean. This value, a percentage of the control response, reduces the variation in results due to differences in control survival and allows the comparison between different tests. T-tests were conducted versus the appropriate control to determine significance at the 95% level.

The degree of agreement among the laboratories for absolute values of survival was first assessed by analysis of variance (ANOVA) for data meeting assumptions of normality, otherwise by a Kruskal-Wallis ANOVA on ranks. In cases where significant differences were found, Tukey pairwise multiple comparison tests were conducted to detect specific differences among the laboratories.

The relative agreement of the laboratories was assessed by ranking the mean survival values within a laboratory. The degree of association of toxicity rankings between laboratories was assessed by Kendall's coefficient of concordance (W) (Siegel and Castellan, 1988). The field sediments were ranked in order of toxicity for each laboratory, with a value of 1.0 assigned to the sediment with the highest survival rate and a value of 4.0 assigned to the sediment with the lowest survival rate. Kendall's W ranges from 0.0 (no degree of association) to 1.0 (perfect association).

Sediment toxicity was defined by two criteria: (1) a statistically significant difference between the sample and control and (2) a minimum percentage difference

between the sample and control. Samples that were significantly different from the control and had a 20% response relative to the control (survival less than 80% of the control) were classified as toxic. This measure of toxicity represents a 90% power to determine statistical significance in survival between control and sample (SAIC 1994). Toxic samples were further classified as moderately toxic (50 to 79% survival) or highly toxic (less than 50% survival). Toxicity classification systems with three or more tiers have been used for amphipod test results as well as for other bioassay species (Long *et al.* 1998).

Mean survival was calculated for each cadmium concentration of the reference toxicant test. Reference toxicant test LC<sub>50</sub> values were calculated with the Spearman-Kärber method. A control chart was prepared by plotting the LC<sub>50</sub> values for the seven laboratories. A test result within two standard deviations of the mean was considered acceptable.

## Results

### Assessment of Laboratory Performance

The participating laboratories successfully met all test acceptability criteria for the amphipod test (Table B1). Each laboratory had nearly 100% survival in the animal collection site control sediment. In addition, each laboratory obtained at least 90% survival in the reference toxicant seawater control (mean=97.6, Table 1). The laboratories reported that all experiments were run within the parameters of the test protocol (including water quality) and that no tests needed to be repeated.

**TABLE B1. Laboratory control performance. Test protocol requirements are from EPA (1994).**

Lab	Sediment Mean Control Survival (%)	Test Conditions Conformed to Protocol?	Reference Toxicant Control Survival (%)
1	99	Yes	100
2	100	Yes	100
3	98	Yes	97
4	96	Yes	90
5	97	Yes	100
6	100	Yes	100
7	97	Yes	97

## Interlaboratory Comparability

### *Reference Toxicant Tests*

A wide range of sensitivity to cadmium was observed between the highest and the lowest values. The LC<sub>50</sub> values ranged from 1.76 to 9.43 µg/L, approximately a factor of five (Table B2). In spite of the overall range, five of the seven laboratory results were within  $\pm 28\%$  of the mean value (5.39 µg/L). The lowest LC<sub>50</sub> value stood out since more than a factor of two separated it from the rest of the group. The LC<sub>50</sub> 95% confidence intervals for the other six laboratories at least partially overlapped one another.

**TABLE B2. Cadmium reference toxicant results obtained during the interlaboratory comparison exercise.**

Lab	LC50 (mg/L)	95% CI
1	1.8	1.6 - 1.9
2	9.4	7.1 - 12.6
3	4.0	3.2 - 5.0
4	5.7	4.6 - 7.0
5	5.9	5.0 - 7.0
6	6.3	4.6 - 8.6
7	4.7	3.9 - 5.7

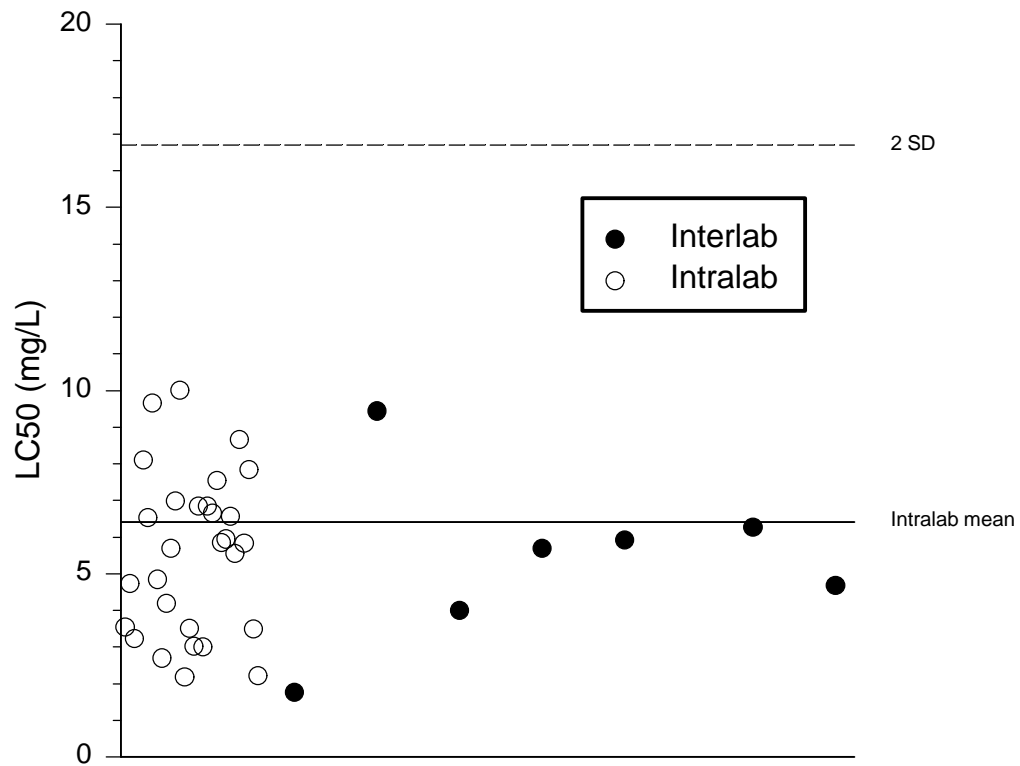
In order to better determine the significance of the reference toxicant variability, the LC<sub>50</sub> values from this interlaboratory test were compared to the results of numerous *Eohaustorius* reference toxicant tests conducted by a single laboratory. The variability and the range of the interlaboratory tests were similar to the intralaboratory variability and range observed in the single laboratory data (Figure B2). The LC50 values of the interlaboratory test fell within two standard deviations of the mean calculated from the intralaboratory test data.

### *Sediment Tests*

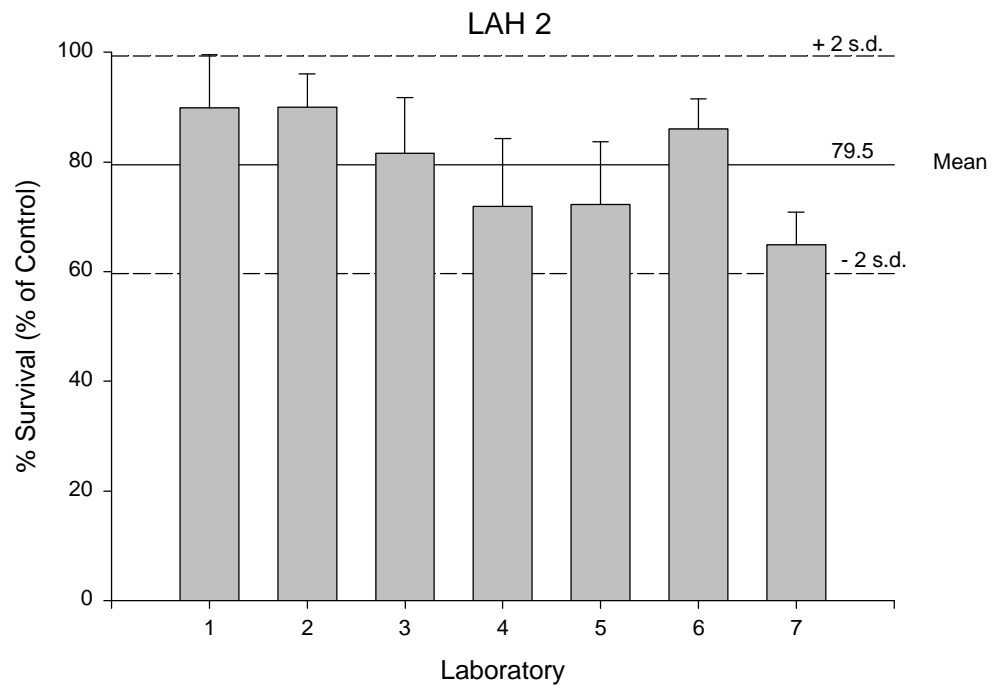
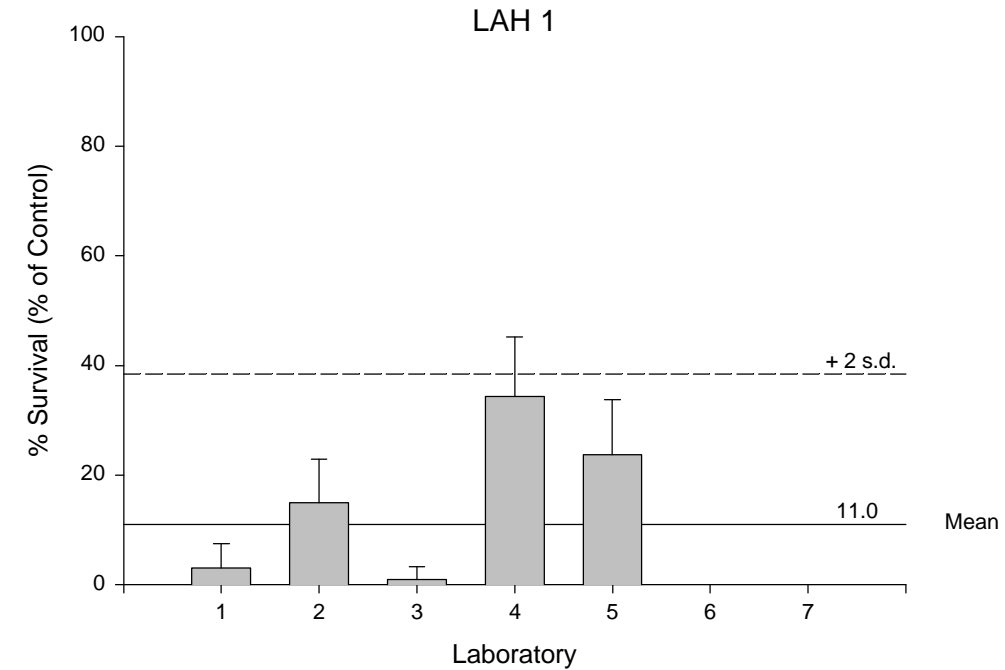
The sediments from the four sampling locations produced a wide range of mean survival values, which was the intent of the study design. Consensus survival means ranged from 11 to 80% (Figure B3). Each of the laboratories was able to statistically discriminate between the control sediment and the field-collected sediments. Overall, 27 of the 28 possible t-tests between control sediments and field sediments were significant ( $p < 0.05$ ). These data demonstrated that each of the laboratories could discriminate between the control sediment and moderately contaminated field sediments.

For each sediment type, all laboratories reported data that fell within two standard deviations of the consensus mean. The standard deviation of the laboratory means ranged

from 9.9 to 16.4 for the four sediment types. Coefficients of variation ranged from 12.4 to 124%. The high CV value (for Station LAH1) was mainly due to low mean survival, which has the effect of inflating the coefficient of variation.

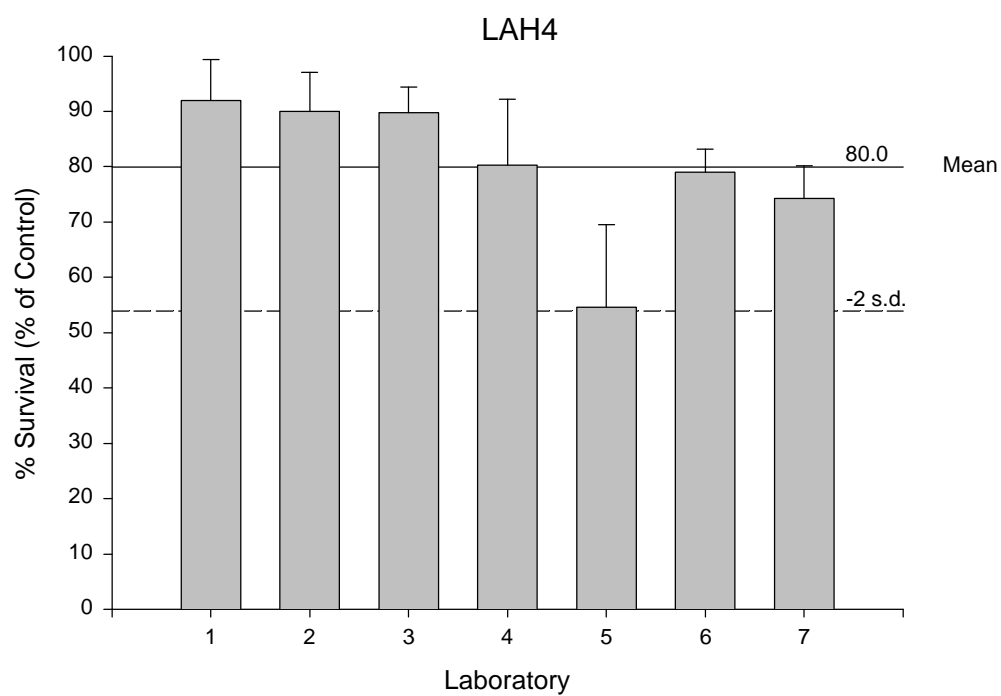
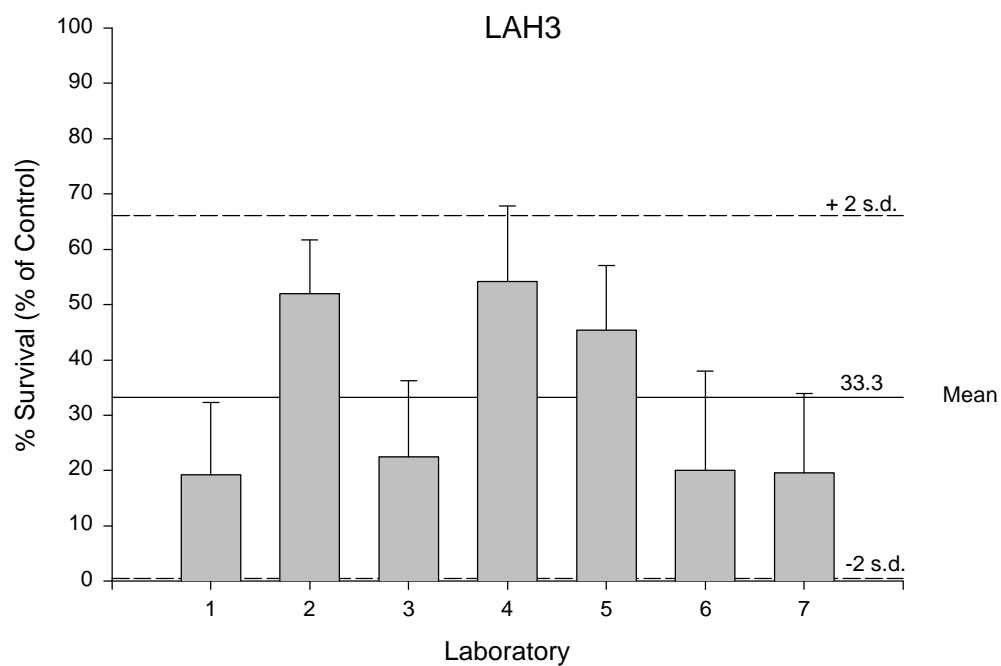


**FIGURE B2. Cadmium reference toxicant median lethal concentrations (LC<sub>50</sub>) for the interlaboratory exercise (filled circles) and for a single laboratory (open circles).**



**FIGURE B3. Survival results for *Eohaustorius estuarius* exposed to field sediments (LAH1-LAH4). Bars represent the mean of 5 replicates tested at each laboratory. Error bars are one standard deviation. Solid reference line is the consensus mean of the seven laboratories. Dashed reference lines are  $\pm 2$  standard deviations from the consensus mean.**





**FIGURE B3. (Continued).I**

Analysis of variance detected statistically significant differences between some of the laboratories for each of the four sediment types ( $p < 0.05$ ). Overall, 25% (21 out of 84) of the possible Tukey pairwise comparisons showed significant differences (Table B3). Two minor trends were observed in the statistical differences. First, Lab 7 had the lowest or second lowest survival rate for each sediment type. However, the Tukey analysis grouped Lab 7 together with at least three of the other laboratories for each sediment type, indicating that the survival results were not markedly different from other laboratories. Second, one laboratory (Lab 5) was different from all the other laboratories for station LAH4. However, this was the only case where a single laboratory was left ungrouped and that three laboratories were grouped together statistically in all other cases. Furthermore, this same laboratory (Lab 5) was the only laboratory that did not differ significantly from *any* other laboratories for field sediments LAH1, LAH2, and LAH3 (indicated in Table B3 by two lines that connect to the other laboratories).

**TABLE B3. Laboratories arranged by order of survival results for each field sediment. Laboratories not significantly different from one another (Tukey pairwise comparison,  $p > 0.05$ ) are connected by solid lines.**

Sediment	Laboratory_Number						
	Highest Survival				Lowest Survival		
LAH1	4	5	2	1	3	6	7
LAH2	2	1	6	3	5	4	7
LAH3	4	2	5	3	6	7	1
LAH4	1	2	3	4	6	7	5

Since the sediments were tested blindly, ranking the four field sediments by survival for each laboratory provided a way to assess the ability of the participating laboratories to distinguish between sediments. Each of the seven laboratories ranked LAH1 and LAH3 as the most toxic and next most toxic, respectively (Table B4). Four of the laboratories ranked the sediments in exactly the same order and a fifth only differed in that LAH2 and LAH4 were tied in the rankings. The Kendall coefficient of concordance was 0.91, indicating a high level of agreement ( $p < 0.01$ ) between laboratories.

**TABLE B4. Rank of field sediments by amphipod survival. 1 = highest survival, 4 = lowest survival.**

Sediment Type	Laboratory						
	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7
Station LAH1	4	4	4	4	4	4	4
Station LAH2	2	1.5	2	2	1	1	2
Station LAH3	3	3	3	3	3	3	3
Station LAH4	1	1.5	1	1	2	2	1

The sediments were classified according to level of toxicity by employing the proposed ratings for the Bight '98 study (non-toxic, moderately toxic, highly toxic). All seven laboratories classified LAH1 sediment as highly toxic (Table B5). All seven laboratories classified LAH3 as toxic, with five classifying it as highly toxic and two as moderately toxic. The classification results were more variable for the two stations with a survival mean of approximately the same value as the moderate toxicity threshold (80%). Three of the seven laboratories classified LAH2 as moderately toxic; four classified it as not toxic. Four laboratories classified LAH4 as moderately toxic, three as not toxic.

**TABLE B5. Comparison of individual laboratory classifications of samples tested. N=nontoxic ( $\geq 80\%$  survival), MT=moderately toxic (50-79% survival, significantly different from the control (t-test,  $p < 0.05$ )), HT=highly toxic ( $< 50\%$  survival, significantly different from the control (t-test,  $p < 0.05$ )).**

	b) Sample Tested			
	LAH1	LAH2	LAH3	LAH4
1	HT	N	HT	N
2	HT	N	MT	N
3	HT	N	HT	N
4	HT	MT	MT	MT
5	HT	MT	HT	MT
6	HT	N	HT	MT
7	HT	MT	HT	MT

## Discussion

All of the participating laboratories successfully completed the interlaboratory comparison exercise. Each participating laboratory demonstrated that it was capable of performing the *Eohaustorius estuarius* 10-d sediment test and associated 96-h reference toxicant test. In addition, each laboratory demonstrated an excellent capability to discriminate statistically between the control sediment and field-collected sediments. These achievements indicated that no major problems occurred with either the general laboratory conditions or the handling techniques employed by the personnel in each laboratory.

The good performance of the laboratories is especially noteworthy considering the fact that many did not have experience with *Eohaustorius estuarius*. This demonstrates that the species is well suited as a test organism in 10-d sediment tests. The test can be learned and applied quickly by laboratories having basic familiarity with marine toxicity tests.

### Interlaboratory Agreement

When multiple laboratories are involved in a study, there is the potential for lower precision in the results than could be obtained by a single laboratory. However, the findings of the current study do not indicate that precision was sacrificed. The interlaboratory range observed for the reference toxicant LC<sub>50</sub> results fell within the intralaboratory range of one very experienced laboratory. The data points produced by multiple laboratories were indistinguishable from the points produced by the single laboratory. This result is noteworthy given that this was the first time some of the laboratories had conducted an amphipod test.

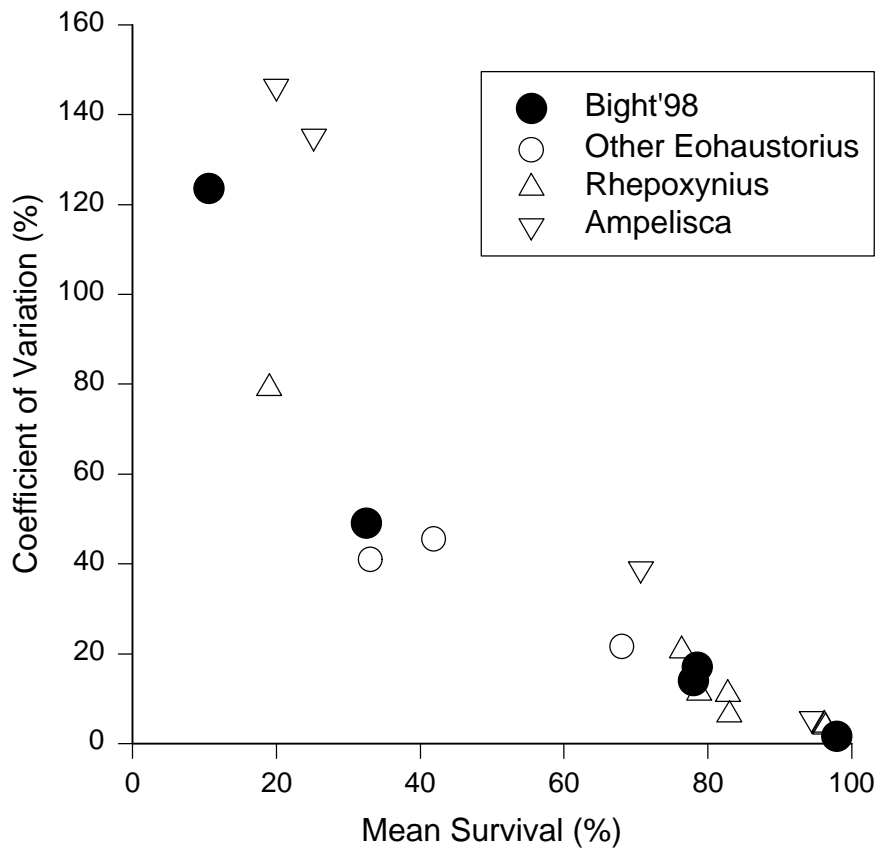
The influence of laboratory conditions and techniques over variations in mean survival values would indicate a finding of consistent bias in the results. However, a strong bias was not observed in the performance of any of the laboratories. Analysis of variance did detect some significant differences between the laboratories for the field-collected sediments, but most (75%) of the differences in pairwise comparisons were not significant. The pairwise comparisons that were significant were distributed among the possible combinations. These results support a finding that the variability observed in the comparison exercise was the result of variability inherent in the test itself.

The laboratories demonstrated an excellent ability to rank field sediments by toxicity. Classification of sediments into the Bight '98 categories also showed reasonably good agreement considering that the grand mean of each of two stations (LAH2 and LAH4) was very close to the classification threshold. It is important to note that the laboratories had 96% agreement on whether the response to the field sediment was statistically different from the control response.

## Comparison to Previous Interlaboratory Studies

The results of our interlaboratory study compare well to a previous interlaboratory study using the same species. Schlekot *et al.* (1995) reported similar findings of agreement for an interlaboratory comparison involving eight laboratories and using multiple dilutions of a contaminated sediment sample. As in our study, the investigators found some significant differences between the mean survival values for individual laboratories. Also, as we did, they found very good agreement in the ability of the laboratories to rank toxicity levels of the sediments tested (Kendall's  $W = 0.93$ ). The largest difference observed between an individual mean survival and the consensus mean survival was 35.9%; in our study, the maximum was 25.4%.

We can compare the relative level of interlaboratory variability observed for the Bight '98 interlaboratory study to previous 10-d amphipod test interlaboratory studies. In the Bight '98 study, the coefficient of variation (CV) ranged from 2% (for the control sediment) to 123% and decreased markedly as mean survival increased. The C.V. for a given level of survival was very similar to that observed in other interlaboratory comparisons (Figure B4). This is an indication that the amount of variability among laboratories observed in the current study was within expected values.



**FIGURE B4.** Interlaboratory variability as a function of mean survival for three commonly applied 10-d amphipod tests. In addition to the current study, data are from Mearns *et al.* (1986) and Schlekot *et al.* (1995).

## Implications for Cooperative Regional Monitoring Programs

Our results show that cooperative toxicity testing can provide data of comparable quality to similar testing performed by a single laboratory. Some improvement may be reasonably expected as laboratories gain more experience working with the test species. Thus, we can expect that the laboratories will perform the *Eohaustorius* tests adequately and have levels of variability equal to or less than those observed in the current study.

The uncertainty associated with the classification of any one station is reduced when many stations are grouped together to provide an assessment of a geographic area, as they are in regional monitoring projects such as Bight '98. Including more stations in a subpopulation decreases the potential that misclassifications will have a significant influence on the assessment of the subpopulation.

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## **APPENDIX C. MICROTOX STORAGE EXPERIMENT**

### **INTRODUCTION**

The time that a water sample is held prior to toxicity test initiation can have a significant effect on toxicity test results and the potential for effects increases with time. Changes to sample constituents are inevitable. For example, sample constituent alteration may occur due to changes in temperature, pH, adsorption of chemicals to holding vessel walls, or biological activity (APHA 1998). Sample holding and preservation techniques can only retard the rate of these changes, which include changes due to biological activity, hydrolysis of chemical compounds, and reduced constituent volatility (APHA 1998).

As part of the Bight'98 regional monitoring survey conducted in July-August, 1998, sediment samples were collected for acid volatile sulfides (AVS) analysis and pore water toxicity testing. The samples were sent to the U.S. EPA Laboratory in Newport, Oregon, for processing and AVS analysis. Pore water was removed by centrifugation. A sub-sample of the pore water was removed, placed in borosilicate glass vials with Teflon lids, and stored at 1.5 – 1.8° C prior to being shipped to the Orange County Sanitation District (OCS D) for toxicity testing. Pore water samples were held until all sediments were received and processed. Some pore water samples were held several weeks prior to shipping. Upon receipt, the samples were frozen at -20° C until thawed for testing using the Microtox™ Rapid Toxicity Testing System (Azur Environmental, Carlsbad, CA). The pore water samples were held for approximately 6 months prior to testing.

Due to concerns over the possible effects of the extended sample holding time on Microtox test results, a storage experiment was conducted. The purpose of this experiment was to determine if sample storage time has a significant effect on pore water toxicity from marine sediment samples as measured using the Microtox™ Rapid Toxicity Testing System.

### **METHODS**

The study was conducted at the OCS D Bioassessment Laboratory (Fountain Valley, CA). Sediment samples for pore water extraction were collected from two locations on March 23, 1999. One was an offshore site (Station 37) not expected to show significant toxicity and the other a Newport Bay site (Station 2137), which was expected to produce a toxic response. The sediment samples were collected using a 0.1 m<sup>2</sup> modified Van Veen grab sampler. Samples were collected using a stainless steel scoop and placed into a HDPE container. Only the top 2 cm of sediment was collected. The samples were kept in wet ice in a cooler on the boat and were subsequently transported to the OCS D for pore water extraction. The pore water was extracted within 24 h of sediment collection. The pore water was extracted by centrifugation (10,000 rpm for 10



minutes), decanted, and stored in glass jars with Teflon-lined lids. Following extraction from the sediments, the pore water samples were kept frozen at -20° C until thawed for testing. Once thawed, the samples were kept refrigerated at 4° C  $\pm$  1° C for the duration of the study.

Testing was conducted using the Microtox™ Comparison Test Method (Microbics Corp. 1995). The Microtox system uses a luminescent bacterium, *Vibrio fischeri* (strain NRRL B-11177), to measure aquatic phase toxicity. The light emitted by the bacteria as a byproduct of metabolic processes is used as a measure of biological activity. Exposure to toxicants alters the metabolic processes, thus affecting light production. Toxicity is assessed by exposing the bacteria to a sample and measuring the light production relative to a control sample. A reduction in light output is assumed to be proportional to the toxicity of a sample.

The Comparison Test Method uses multiple replicates of the sample at a single concentration (in this case, 100% sample) and compares light output against a control (clean seawater) to measure relative acute toxicity of the sample. Light readings are taken at time zero, five, and fifteen minutes. The time zero light measurement is taken as the initial reading to which subsequent light measurements are compared. Different toxic constituents can affect the bacteria at different rates. Some toxicants (e.g., phenol) cause an immediate reduction in light production, while others act over a longer time period. Taking light measurements at both times is recommended (Microbics Corp. 1992).

In this study, three replicates of sample were compared against three replicates of control water. The control water was clean seawater obtained from the Kerckhoff Marine Laboratory, operated by the California Institute of Technology (Corona Del Mar, CA). The seawater was filtered through a 2  $\mu$ m filter and stored at 4° C  $\pm$  1° C. The test was conducted using the Microtox® M500 Analyzer, at a temperature of 15° C. Testing was initiated one-week from pore water extraction and continued at one-week intervals for four weeks and then monthly for four additional months.

## RESULTS

In general, the magnitude and pattern of changes in toxicity over time were similar for both stations and for both 5 and 15-minute measurements. Neither station showed toxicity at the initial time period (one-week); luminescence was greater than or approximately equal to the control luminescence (Table C1, Figure C1).

A marked decrease was found in light production relative to the control from week one to week two for both samples. Toxicity then varied within a relatively narrow range through a holding time of one month. At the two-month testing, light production increased in the Station 37 sample to 93% of the control. However, the sample from Station 2137 showed no significant change.

After three and four months of storage, the light production of the Station 37 sample was greater than that of the control. However, light production decreased to below the toxic threshold of 95% of the control at the final testing period (five months). The Station 2137 sample had a sharp increase in light production at the three-month sampling interval, but was still below the toxic threshold. Light production subsequently declined for Station 2137 (four months) and then remained approximately unchanged at five months.

## **DISCUSSION**

The results of this study indicate that toxicity tends to increase approximately 20% with a storage time of two weeks or greater. Toxicity then decreases at two to three months' storage time, but increases again at four months. The cause of the variability was not identified.

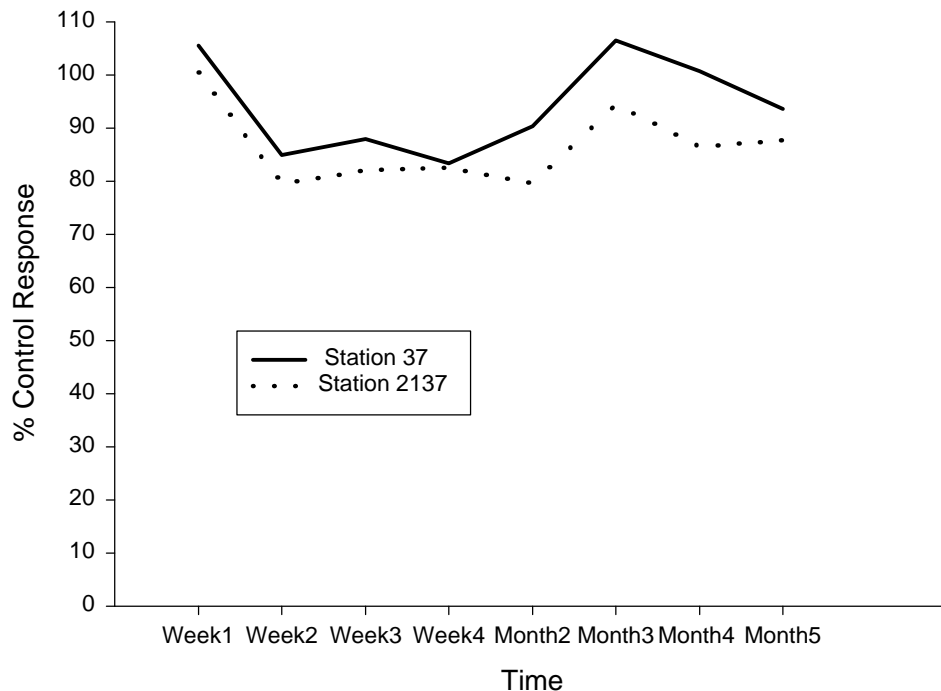
In this study, significant changes were seen in toxicity after samples were held for only two-weeks. Some of the samples taken in the Bight '98 project were held in the processing laboratory up to four-weeks before delivery to the testing laboratory. All samples received by the testing laboratory were held frozen for approximately six-months prior to toxicity testing. The results of this storage experiment indicate Microtox test results would have been compromised even if testing was initiated immediately upon receipt of the samples due to the holding time in the processing laboratory.

The porewater samples in the Bight '98 study were held frozen at  $-20^{\circ}\text{C}$  by the testing laboratory prior to testing. While, there is no one method of preservation that satisfactorily prevents the degradation of all constituents in a water sample, the effect of pore water freezing relative to Microtox test results has not been investigated. This method of preservation may have also altered the chemical nature of the sample and thus the toxicity test results.

The results of this storage study on the toxicity of marine pore water toxicity, as measured by the Microtox<sup>TM</sup> system, indicate that the length of time the Bight '98 sediment porewater samples were held prior to testing was sufficient to significantly affect Microtox toxicity test results. Therefore, the results of Microtox testing of sediment porewater in the Bight'98 project were excluded from regional toxicity assessments.

**Table C1. Results of sample storage time on pore water toxicity measured by Microtox. Values reported are light production expressed as a percentage of the seawater control response for either a 5 or 15 minute exposure.**

Storage Time	Date Tested	Station 37		Station 2137	
		5 min.	15 min.	5 min.	15 min.
1 week	3/31/99	105.5	105.9	100.5	99.1
2 weeks	4/07/99	84.9	81.6	79.5	72.3
3 weeks	4/14/99	87.9	87.0	82.0	78.8
4 weeks	4/20/99	83.4	81.7	82.5	77.7
2 months	5/14/99	90.3	93.3	79.6	79.1
3 months	6/15/99	106.5	109.3	94.4	92.7
4 months	7/19/99	100.7	106.0	86.5	79.8
5 months	8/17/99	93.6	92.3	87.7	80.9



**Figure C1. Change of toxicity over the five-month study period. Fifteen minute reading values are shown.**

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## APPENDIX D. TEST RESULTS BY STATION

Station	Longitude	Latitude	Strata <sup>a</sup>	Depth (m)	Location	Amphipod (%)	Test Response <sup>b</sup>			
							QwikSed (%)	HRGS (µg/g)	Fines (%)	TOC (%)
2268	119.8000	34.4007	Bath-120	31	Goleta	89.0	100.0	22.8	27.0	0.68
2381	118.7693	34.0025	Bath-120	40	Los Angeles Co.	103.2	97.6	4.8	61.9	0.94
2384	118.5039	33.9299	Bath-120	47	Los Angeles Co.	100.0	na	10.7	49.8	0.77
2387	118.4522	33.8483	Bath-120	75	Los Angeles Co.	101.0	100.0	14.4	45.1	0.88
2394	118.2491	33.6511	Bath-120	42	Los Angeles Co.	101.0	88.7	2.8	16.5	0.50
2396	118.1494	33.6481	Bath-120	31	Orange Co.	90.6	na	2.1	16.8	0.32
2398	118.1430	33.6193	Bath-120	41	Orange Co.	97.9	na	2.8	15.6	0.26
2400	118.0954	33.6033	Bath-120	52	Orange Co.	93.8	97.7	2.3	23.3	0.29
2401	117.9575	33.5917	Bath-120	41	Orange Co.	100.0	na	2.1	46.4	0.53
2403	117.8029	33.5187	Bath-120	89	San Diego Co.	96.4	84.7	2.2	75.4	0.86
2405	117.5586	33.2946	Bath-120	58	San Diego Co.	91.8	100.0	3.1	67.0	0.78
2407	117.5237	33.2603	Bath-120	58	San Diego Co.	93.8	na	2.3	58.5	0.69
2408	117.3618	33.1051	Bath-120	83	San Diego Co.	92.8	100.0	3.5	59.3	0.66
2410	117.3546	32.7722	Bath-120	87	San Diego Co.	84.5	100.0	3.2	46.9	1.26
2411	117.3430	32.7526	Bath-120	89	San Diego Co.	78.4	na	4.5	52.9	0.68
2412	117.2963	32.7214	Bath-120	62	San Diego Co.	99.0	100.0	4.1	46.6	0.67
2413	117.2784	32.6905	Bath-120	49	San Diego Co.	95.9	100.0	3.8	28.7	0.48
2418	117.3148	32.5953	Bath-120	100	San Diego Co.	100.0	na	6.1	24.5	0.48
2419	117.2636	32.5894	Bath-120	56	San Diego Co.	101.0	100.0	7.0	10.7	0.39
2356	120.0739	34.4477	Bath-120	44	Santa Barbara Co.	97.8	na	35.0	30.5	0.74
2357	119.9408	34.4064	Bath-120	57	Santa Barbara Co.	74.7	100.0	77.0	74.3	1.82
2358	119.9730	34.3968	Bath-120	75	Santa Barbara Co.	76.8	100.0	23.0	42.3	1.15
2360	119.8752	34.3940	Bath-120	46	Santa Barbara Co.	69.7	100.0	902.9	49.4	2.82
2362	119.6681	34.3683	Bath-120	55	Santa Barbara Co.	84.0	100.0	12.6	71.6	1.41
2363	119.6281	34.3556	Bath-120	45	Santa Barbara Co.	97.0	100.0	2.6	0.6	0.12
2364	119.6568	34.3434	Bath-120	14	Santa Barbara Co.	94.0	na	5.6	21.6	0.33
2365	119.5623	34.3447	Bath-120	45	Santa Barbara Co.	73.0	100.0	9.5	55.1	0.75
2366	119.6109	34.3309	Bath-120	57	Santa Barbara Co.	75.8	100.0	15.9	46.1	0.72
2367	119.5332	34.3168	Bath-120	61	Santa Barbara Co.	92.0	na	7.2	98.9	1.52
2368	119.4633	34.3014	Bath-120	45	Ventura Co.	68.0	na	15.0	98.5	1.39
2369	119.4252	34.3040	Bath-120	33	Ventura Co.	72.0	100.0	10.9	96.0	1.21
2371	119.5101	34.2641	Bath-120	76	Ventura Co.	80.0	na	18.5	94.8	1.58
2372	119.6020	34.2399	Bath-120	107	Ventura Co.	90.9	100.0	15.3	40.5	0.63
2374	119.4911	34.1880	Bath-120	98	Ventura Co.	71.7	na	17.1	75.7	1.42
2132	119.2272	34.1549	Bath-30	3	Channel Is. Harbor	101.1	100.0	2.8	2.0	0.06
2377	118.9403	34.0389	Bath-30	19	Los Angeles Co.	95.9	na	2.6	11.3	0.18
2378	118.9225	34.0325	Bath-30	27	Los Angeles Co.	94.8	100.0	2.3	15.3	0.23
2379	118.8368	34.0192	Bath-30	17	Los Angeles Co.	68.0	na	1.4	5.1	0.18
2380	118.7601	34.0183	Bath-30	20	Los Angeles Co.	94.8	na	4.6	29.4	0.64
2382	118.5926	34.0231	Bath-30	23	Los Angeles Co.	97.9	na	6.8	43.4	0.71

Station	Longitude	Latitude	Strata <sup>a</sup>	Depth (m)	Location	Test Response <sup>b</sup>				
						Amphipod (%)	QwikSed (%)	HRGS (µg/g)	Fines (%)	TOC (%)
2383	118.5133	34.0118	Bath-30	10	Los Angeles Co.	97.9	100.0	5.7	20.7	0.31
2385	118.4572	33.9068	Bath-30	25	Los Angeles Co.	97.9	100.0	4.2	7.1	0.15
2386	118.4245	33.8781	Bath-30	16	Los Angeles Co.	101.1	na	6.5	6.8	0.16
2388	118.1487	33.7483	Bath-30	13	Los Angeles Co.	87.8	92.7	4.8	65.5	0.85
2389	118.3218	33.7095	Bath-30	20	Los Angeles Co.	103.1	na	3.1	6.4	1.75
2390	118.2647	33.6935	Bath-30	25	Los Angeles Co.	100.0	93.8	12.6	60.3	1.50
2391	118.1378	33.7095	Bath-30	19	Los Angeles Co.	97.9	na	8.1	51.3	0.49
2392	118.1554	33.7051	Bath-30	21	Los Angeles Co.	102.1	na	2.7	19.8	0.26
2393	118.0883	33.6829	Bath-30	17	Orange Co.	96.9	na	1.0	1.7	0.07
2395	118.0541	33.6727	Bath-30	13	Orange Co.	102.1	na	1.0	8.3	0.12
2397	118.1260	33.6501	Bath-30	30	Orange Co.	101.0	100.0	3.1	24.4	0.56
2399	117.9948	33.6347	Bath-30	12	Orange Co.	101.0	na	0.8	13.2	0.14
2402	117.8302	33.5574	Bath-30	30	San Diego Co.	95.4	100.0	3.9	50.2	1.19
2404	117.6589	33.4221	Bath-30	21	San Diego Co.	96.9	na	1.7	36.5	0.27
2406	117.4719	33.2814	Bath-30	11	San Diego Co.	87.6	100.0	1.6	28.5	0.23
2409	117.2820	33.0041	Bath-30	7	San Diego Co.	94.8	na	1.0	0.6	0.07
2414	117.1898	32.6770	Bath-30	9	San Diego Co.	99.0	na	1.6	0.3	0.15
2415	117.1823	32.6587	Bath-30	14	San Diego Co.	100.0	na	0.9	6.4	0.10
2417	117.2146	32.6250	Bath-30	24	San Diego Co.	98.0	na	1.1	1.5	0.23
2354	120.3128	34.4589	Bath-30	17	Santa Barbara Co.	103.3	na	5.2	16.4	0.34
2355	120.3367	34.4519	Bath-30	16	Santa Barbara Co.	101.4	84.4	9.7	12.0	0.25
2359	119.8648	34.3987	Bath-30	26	Santa Barbara Co.	94.9	na	89.8	20.5	0.66
2361	119.5701	34.3994	Bath-30	13	Santa Barbara Co.	98.0	na	5.9	13.7	0.19
2370	119.3618	34.2986	Bath-30	11	Santa Barbara Co.	96.8	na	2.8	20.6	0.28
2373	119.3851	34.2208	Bath-30	28	Ventura Co.	96.0	100.0	8.1	71.5	0.54
2375	119.3554	34.1880	Bath-30	27	Ventura Co.	100.0	100.0	6.7	62.4	0.45
2376	119.3464	34.1788	Bath-30	26	Ventura Co.	96.9	100.0	4.2	50.8	0.36
2189	118.5596	33.9533	LPOTW	63	Hyperion WWTP	na	na	9.0	19.0	0.80
2190	118.5287	33.9479	LPOTW	50	Hyperion WWTP	104.3	100.0	13.9	33.4	0.64
2191	118.5607	33.9370	LPOTW	98	Hyperion WWTP	90.6	86.0	15.7	17.8	0.77
2192	118.5191	33.9445	LPOTW	48	Hyperion WWTP	103.2	5.0	6.1	35.2	0.58
2194	118.5215	33.9204	LPOTW	57	Hyperion WWTP	na	na	12.7	42.0	0.76
2195	118.5250	33.9109	LPOTW	59	Hyperion WWTP	103.2	1.9	17.8	43.1	0.78
2196	118.5534	33.9033	LPOTW	72	Hyperion WWTP	na	na	7.8	19.0	1.79
2197	118.5008	33.9039	LPOTW	53	Hyperion WWTP	104.3	100.0	9.8	55.4	0.85
2198	118.5238	33.8872	LPOTW	57	Hyperion WWTP	na	na	5.9	22.0	1.35
2199	118.5349	33.8816	LPOTW	52	Hyperion WWTP	96.9	50.6	7.4	15.8	1.53
2200	118.4290	33.7479	LPOTW	51	JWPCP	na	na	15.9	51.0	3.64
2201	118.3924	33.7207	LPOTW	86	JWPCP	98.9	100.0	49.4	64.7	2.75
2202	118.3725	33.7221	LPOTW	48	JWPCP	89.2	100.0	16.7	72.2	2.06
2204	118.3390	33.6969	LPOTW	76	JWPCP	na	na	58.4	66.0	3.09
2205	118.3058	33.6769	LPOTW	64	Orange Co. WWTP	101.1	100.0	15.2	40.6	1.10
2207	118.0659	33.6126	LPOTW	33	Orange Co. WWTP	na	na	1.0	6.0	0.13

Station	Longitude	Latitude	Strata <sup>a</sup>	Depth (m)	Location	Test Response <sup>b</sup>				
						Amphipod (%)	QwikSed (%)	HRGS (µg/g)	Fines (%)	TOC (%)
2208	118.0565	33.6022	LPOTW	37	Orange Co. WWTP	96.9	100.0	1.7	19.0	0.26
2209	118.0629	33.5920	LPOTW	65	Orange Co. WWTP	103.1	0.2	3.7	25.2	0.43
2210	118.0412	33.5902	LPOTW	45	Orange Co. WWTP	na	na	2.2	24.0	0.40
2211	118.0100	33.5880	LPOTW	41	Orange Co. WWTP	na	na	2.1	26.0	0.44
2212	117.9893	33.5901	LPOTW	40	Orange Co. WWTP	101.0	100.0	2.5	37.6	0.39
2213	117.9778	33.5715	LPOTW	60	Orange Co. WWTP	na	na	1.0	11.0	0.23
2214	117.3011	32.6899	LPOTW	74	Point Loma WWTP	88.7	100.0	3.3	52.5	0.74
2217	117.3106	32.6627	LPOTW	86	Point Loma WWTP	101.0	nv	3.3	46.8	0.59
2218	117.2798	32.6664	LPOTW	59	Point Loma WWTP	56.7	100.0	3.5	44.6	0.65
2219	117.3314	32.6548	LPOTW	106	Point Loma WWTP	na	na	6.4	36.0	1.01
2220	117.2800	32.6597	LPOTW	60	Point Loma WWTP	na	na	8.6	40.0	0.49
2134	118.0622	33.7154	Marina	3	Anaheim Bay	77.3	79.8	18.4	77.7	2.99
2129	119.2278	34.1732	Marina	5	Channel Is. Harbor	55.7	85.1	18.6	79.1	2.44
2130	119.2233	34.1718	Marina	3	Channel Is. Harbor	39.8	86.6	23.1	90.7	2.54
2131	119.2247	34.1628	Marina	3	Channel Is. Harbor	105.4	79.1	8.9	90.2	1.28
2149	117.7042	33.4616	Marina	4	Dana Point Harbor	88.7	100.0	5.4	31.7	0.67
2150	117.7043	33.4624	Marina	2	Dana Point Harbor	97.9	100.0	4.2	42.3	0.69
2151	117.6990	33.4598	Marina	5	Dana Point Harbor	99.0	100.0	16.9	76.8	1.43
2421	118.2409	33.7663	Marina	14	Los Angeles Harbor	85.7	100.0	60.0	84.6	1.84
2443	118.4562	33.9820	Marina	3	Marina del Rey Harbor	65.9	100.0	36.7	84.7	1.64
2444	118.4486	33.9830	Marina	3	Marina del Rey Harbor	79.1	100.0	10.4	90.3	1.59
2445	118.4554	33.9776	Marina	3	Marina del Rey Harbor	70.3	100.0	9.6	79.8	1.42
2446	118.4422	33.9775	Marina	3	Marina del Rey Harbor	102.2	94.4	10.8	58.5	1.07
2447	118.4478	33.9766	Marina	3	Marina del Rey Harbor	103.3	100.0	14.9	77.3	1.42
2448	118.4468	33.9695	Marina	5	Marina del Rey Harbor	107.7	45.7	40.5	57.3	1.35
2449	118.4550	33.9657	Marina	4	Marina del Rey Harbor	87.9	99.2	95.0	61.0	2.31
2423	117.2491	32.7807	Marina	3	Mission Bay	97.9	59.3	6.2	42.2	1.18
2424	117.2475	32.7665	Marina	6	Mission Bay	100.0	100.0	2.2	7.2	0.22
2425	117.2356	32.7673	Marina	4	Mission Bay	97.9	100.0	24.3	69.5	3.48
2136	117.9272	33.6189	Marina	6	Newport Harbor	32.2	100.0	80.7	87.6	1.96
2137	117.9239	33.6130	Marina	4	Newport Harbor	37.4	39.6	22.1	83.7	2.21
2138	117.9141	33.6141	Marina	5	Newport Harbor	9.0	82.1	6.7	93.0	1.46
2141	117.9022	33.6114	Marina	3	Newport Harbor	59.4	100.0	14.3	88.6	1.49
2142	117.9100	33.6077	Marina	3	Newport Harbor	21.9	25.3	10.1	89.6	1.18
2143	117.9063	33.6070	Marina	3	Newport Harbor	36.1	81.1	8.5	91.6	1.27
2144	117.9006	33.6075	Marina	3	Newport Harbor	70.8	79.1	11.3	81.3	1.03
2145	117.8887	33.6038	Marina	3	Newport Harbor	65.6	72.4	7.9	88.3	1.33
2146	117.8874	33.6019	Marina	4	Newport Harbor	85.4	100.0	9.5	64.1	0.95
2147	117.8927	33.6012	Marina	3	Newport Harbor	52.1	92.3	12.4	86.1	1.14
2148	117.8797	33.5944	Marina	7	Newport Harbor	89.6	79.4	4.8	21.3	0.54
2221	117.2051	32.7279	Marina	4	San Diego Harbor	82.3	100.0	20.3	68.7	0.86
2222	117.2259	32.7188	Marina	5	San Diego Harbor	82.3	100.0	9.4	74.2	0.99
2223	117.2305	32.7154	Marina	4	San Diego Harbor	88.5	80.5	16.2	76.2	1.11
2224	117.2341	32.7131	Marina	5	San Diego Harbor	96.9	100.0	11.3	40.7	0.62

Station	Longitude	Latitude	Strata <sup>a</sup>	Depth (m)	Location	Test Response <sup>b</sup>				
						Amphipod (%)	QwikSed (%)	HRGS (µg/g)	Fines (%)	TOC (%)
2225	117.2302	32.7134	Marina	4	San Diego Harbor	88.5	72.2	14.6	58.1	1.03
2226	117.2317	32.7111	Marina	5	San Diego Harbor	86.5	98.3	19.7	91.4	1.73
2227	117.2080	32.7237	Marina	9	San Diego Harbor	97.9	76.0	12.0	51.1	0.93
2228	117.1782	32.7241	Marina	5	San Diego Harbor	101.0	100.0	47.1	45.9	0.73
2438	117.1017	32.6223	Marina	3	San Diego Harbor	79.6	84.5	4.4	67.7	0.92
2128	119.2594	34.2461	Marina	3	Ventura Harbor	80.0	87.8	17.7	95.4	1.74
2229	117.1760	32.7090	Other	12	San Diego Harbor	97.9	1.3	15.6	43.1	0.93
2230	117.1787	32.7025	Other	4	San Diego Harbor	65.7	nv	5.8	11.8	0.20
2231	117.1566	32.6947	Other	13	San Diego Harbor	93.8	82.1	9.1	31.8	0.64
2233	117.1518	32.6858	Other	9	San Diego Harbor	99.0	94.0	19.4	37.9	0.45
2235	117.1369	32.6408	Other	4	San Diego Harbor	71.4	100.0	5.0	43.3	0.64
2238	117.1287	32.6254	Other	3	San Diego Harbor	86.7	52.5	3.4	57.6	0.97
2239	117.1451	32.6824	Other	11	San Diego Harbor	100.0	66.9	15.9	34.6	0.72
2240	117.1541	32.6675	Other	3	San Diego Harbor	88.8	86.2	4.7	44.3	0.55
2241	117.1365	32.6703	Other	4	San Diego Harbor	97.9	69.1	7.7	19.4	0.52
2242	117.1498	32.6650	Other	4	San Diego Harbor	91.8	92.2	5.5	32.6	0.74
2243	117.1427	32.6645	Other	4	San Diego Harbor	95.9	96.0	5.2	35.1	0.49
2244	117.1318	32.6597	Other	3	San Diego Harbor	100.0	51.9	3.5	21.2	0.29
2245	117.1427	32.6508	Other	4	San Diego Harbor	65.7	100.0	5.4	59.6	0.78
2247	117.1247	32.6423	Other	3	San Diego Harbor	89.8	32.8	5.0	43.3	0.58
2249	117.1281	32.6213	Other	3	San Diego Harbor	75.5	100.0	10.4	73.9	1.35
2433	117.2092	32.7224	Other	9	San Diego Harbor	96.9	61.3	18.5	72.3	1.17
2434	117.1836	32.7249	Other	3	San Diego Harbor	101.0	92.3	13.4	44.2	0.71
2435	117.2229	32.7115	Other	12	San Diego Harbor	102.1	68.4	6.1	49.5	0.55
2436	117.1831	32.7150	Other	11	San Diego Harbor	100.0	27.8	25.2	55.9	1.36
2164	118.0826	33.7303	Other	3	Anaheim Bay	93.8	100.0	9.9	22.1	0.42
2152	118.1627	33.7593	Other	6	Los Angeles Harbor	101.0	92.9	7.2	68.2	0.81
2153	118.1578	33.7535	Other	5	Los Angeles Harbor	96.9	100.0	8.9	37.0	0.64
2154	118.1559	33.7490	Other	11	Los Angeles Harbor	96.9	100.0	6.5	34.4	0.50
2155	118.1675	33.7433	Other	12	Los Angeles Harbor	88.7	100.0	19.6	87.3	1.82
2156	118.1713	33.7400	Other	13	Los Angeles Harbor	84.5	100.0	19.6	88.9	1.73
2157	118.1532	33.7423	Other	10	Los Angeles Harbor	77.3	100.0	9.2	47.9	0.65
2158	118.2086	33.7283	Other	21	Los Angeles Harbor	90.7	68.2	16.2	82.1	1.63
2159	118.2104	33.7225	Other	15	Los Angeles Harbor	80.4	90.3	19.8	71.3	1.98
2160	118.2047	33.7236	Other	14	Los Angeles Harbor	84.5	42.2	14.8	73.9	2.27
2161	118.2017	33.7235	Other	14	Los Angeles Harbor	90.7	72.8	12.8	77.7	2.26
2162	118.2417	33.7135	Other	14	Los Angeles Harbor	90.3	100.0	5.6	90.0	1.15
2163	118.1668	33.7279	Other	15	Los Angeles Harbor	89.7	97.3	11.1	75.5	1.36
2167	118.1577	33.7356	Other	13	Los Angeles Harbor	88.7	97.6	16.8	86.6	1.68
2168	118.2506	33.7119	Other	27	Los Angeles Harbor	80.2	100.0	5.3	80.7	1.85
2426	118.2314	33.7342	Other	11	Los Angeles Harbor	99.0	100.0	4.7	38.2	0.46
2427	118.2355	33.7309	Other	9	Los Angeles Harbor	52.0	100.0	6.2	89.4	1.47
2428	118.2585	33.7188	Other	23	Los Angeles Harbor	67.7	100.0	3.5	99.9	1.09
2188	118.0888	33.7338	Port	11	Anaheim Bay	85.6	87.2	31.2	85.7	1.84



Station	Longitude	Latitude	Strata <sup>a</sup>	Depth (m)	Location	Test Response <sup>b</sup>				
						Amphipod (%)	QwikSed (%)	HRGS (µg/g)	Fines (%)	TOC (%)
2169	118.2784	33.7682	Port	11	Los Angeles Harbor	79.4	91.1	181.5	83.2	2.54
2170	118.2560	33.7643	Port	13	Los Angeles Harbor	16.5	99.6	128.4	77.7	2.96
2172	118.2427	33.7492	Port	11	Los Angeles Harbor	78.4	89.8	45.7	55.0	0.93
2173	118.2377	33.7473	Port	12	Los Angeles Harbor	89.7	100.0	14.8	70.4	0.96
2174	118.2666	33.7343	Port	3	Los Angeles Harbor	97.8	100.0	24.6	42.6	1.09
2175	118.2267	33.7409	Port	11	Los Angeles Harbor	85.6	83.6	12.7	71.5	0.87
2176	118.2611	33.7310	Port	18	Los Angeles Harbor	60.2	100.0	3.2	95.8	1.09
2177	118.2426	33.7348	Port	16	Los Angeles Harbor	77.3	100.0	3.3	84.0	1.15
2178	118.2710	33.7280	Port	14	Los Angeles Harbor	91.8	100.0	23.3	87.1	2.53
2179	118.2103	33.7390	Port	26	Los Angeles Harbor	78.4	100.0	13.7	88.4	1.39
2182	118.2623	33.7238	Port	19	Los Angeles Harbor	87.6	100.0	2.7	75.4	0.68
2184	118.2691	33.7206	Port	15	Los Angeles Harbor	91.8	98.3	20.0	88.4	2.83
2185	118.1998	33.7332	Port	23	Los Angeles Harbor	89.7	100.0	21.6	82.9	1.50
2186	118.1930	33.7314	Port	15	Los Angeles Harbor	88.7	96.1	9.7	52.5	0.81
2187	118.1840	33.7312	Port	15	Los Angeles Harbor	82.5	96.9	7.5	50.1	0.90
2430	118.2245	33.7691	Port	18	Los Angeles Harbor	66.3	77.5	19.9	83.7	1.56
2431	118.2241	33.7534	Port	12	Los Angeles Harbor	94.8	100.0	30.5	77.6	1.05
2432	118.2304	33.7508	Port	16	Los Angeles Harbor	87.6	77.3	22.2	93.1	1.45
2251	117.1621	32.7023	Port	9	San Diego Harbor	76.0	100.0	84.9	73.0	1.99
2252	117.1529	32.6919	Port	11	San Diego Harbor	104.2	100.0	18.2	16.7	0.59
2253	117.1381	32.6881	Port	8	San Diego Harbor	88.9	96.1	67.9	66.5	1.57
2254	117.1632	32.6773	Port	5	San Diego Harbor	98.0	100.0	97.1	35.6	0.66
2255	117.1294	32.6780	Port	11	San Diego Harbor	96.9	100.0	36.6	59.8	1.18
2256	117.1359	32.6769	Port	8	San Diego Harbor	100.0	100.0	16.6	65.9	1.26
2257	117.1341	32.6768	Port	9	San Diego Harbor	90.8	100.0	34.8	77.8	1.63
2258	117.1321	32.6759	Port	11	San Diego Harbor	91.8	100.0	59.5	71.9	1.44
2259	117.1247	32.6702	Port	11	San Diego Harbor	96.9	97.6	31.3	69.0	1.24
2260	117.1300	32.6672	Port	4	San Diego Harbor	73.5	83.1	5.5	27.7	0.51
2262	117.1229	32.6515	Port	10	San Diego Harbor	78.5	86.2	27.9	74.8	1.64
2263	117.1760	32.7161	Port	13	San Diego Harbor	88.2	100.0	115.1	73.6	1.28
2264	117.1328	32.6854	Port	10	San Diego Harbor	89.8	-99.0	123.0	73.4	1.99
2265	117.1403	32.6839	Port	11	San Diego Harbor	84.9	100.0	14.0	13.8	0.35
2439	117.1895	32.7261	Port	3	San Diego Harbor	84.4	78.6	26.4	53.2	1.03
2440	117.1748	32.7185	Port	10	San Diego Harbor	103.1	na	19.1	37.4	0.50
2441	117.2380	32.6912	Port	16	San Diego Harbor	87.9	84.6	32.3	79.9	1.97
2442	117.2371	32.6892	Port	13	San Diego Harbor	80.8	79.3	72.4	77.6	1.99
2302	117.7764	33.5100	River	50	Ballona Creek	88.2	na	4.2	78.4	1.02
2303	117.7819	33.5187	River	48	Ballona Creek	90.3	na	3.6	71.5	0.83
2304	117.7696	33.5213	River	14	Ballona Creek	93.5	na	1.3	1.1	0.08
2305	118.4711	33.9757	River	8	Ballona Creek	104.3	na	3.8	9.9	0.18
2306	118.4536	33.9359	River	14	Ballona Creek	100.0	na	1.8	1.7	0.17
2307	118.4767	33.9623	River	15	Ballona Creek	101.1	na	3.9	15.2	0.21
2311	118.1850	33.7555	River	14	Los Angeles River	61.9	na	55.1	71.1	2.20
2312	118.6649	34.0202	River	30	Malibu Creek	101.0	na	12.8	61.0	1.26

Station	Longitude	Latitude	Strata <sup>a</sup>	Depth (m)	Location	Test Response <sup>b</sup>				
						Amphipod (%)	QwikSed (%)	HRGS (µg/g)	Fines (%)	TOC (%)
2314	118.6825	34.0271	River	11	Malibu Creek	102.1	na	6.2	14.6	0.24
2308	119.0902	34.0831	River	74	Mugu Lagoon	63.9	na	3.4	59.6	1.22
2310	119.0891	34.0916	River	18	Mugu Lagoon	92.8	na	5.1	36.3	0.25
2315	117.2697	32.7695	River	19	San Diego River	100.0	na	1.1	0.0	0.07
2317	117.2757	32.7671	River	23	San Diego River	100.0	na	1.2	1.0	0.08
2318	118.1269	33.7237	River	12	San Gabriel River	94.8	na	4.3	11.7	0.22
2319	118.1435	33.7371	River	12	San Gabriel River	94.8	na	12.5	66.6	0.99
2320	118.1215	33.7330	River	7	San Gabriel River	96.9	na	10.8	6.4	0.15
2321	118.1335	33.7293	River	12	San Gabriel River	88.7	na	8.8	26.8	0.42
2325	117.9872	33.6277	River	13	San Gabriel River	97.9	na	1.2	16.0	0.14
2326	117.9562	33.6227	River	8	San Gabriel River	92.8	na	2.1	16.8	0.08
2328	119.2877	34.2191	River	15	Santa Clara River	82.8	na	3.7	40.2	0.37
2329	119.2764	34.2501	River	11	Santa Clara River	100.0	100.0	3.9	0.5	0.18
2330	119.2808	34.2399	River	12	Santa Clara River	93.8	na	4.0	34.8	0.38
2331	119.2887	34.2456	River	16	Santa Clara River	92.5	na	4.1	91.6	0.92
2339	119.2848	34.2523	River	15	Santa Clara River	92.8	na	4.1	72.8	0.73
2335	117.1548	32.5447	River	18	Tijuana River	101.0	na	1.6	17.2	0.16
2338	119.3413	34.2699	River	19	Ventura River	100.0	na	3.4	24.0	0.31
2340	119.3416	34.2651	River	20	Ventura River	104.3	na	3.4	15.1	0.20
2450	118.1994	33.7603	River-grad	5	Los Angeles River	7.2	na	88.5	70.6	3.44
2451	118.1742	33.7519	River-grad	12	Los Angeles River	64.9	na	33.8	64.7	0.97
2453	117.9749	33.6279	River-grad	11	San Gabriel River	95.4	na	1.2	30.3	0.12
2454	117.7553	33.5090	River-grad	10	San Juan Creek	86.6	na	2.3	18.9	0.09
2289	117.3585	33.1025	SPOTW	78	Encina	96.8	100.0	3.9	62.5	0.66
2290	117.3588	33.1162	SPOTW	187	Encina	87.2	100.0	8.3	81.0	1.94
2266	119.8217	34.3982	SPOTW	41	Goleta	na	na	51.4	52.0	1.43
2267	119.8254	34.4060	SPOTW	14	Goleta	92.0	100.0	14.5	16.1	0.34
2301	119.8330	34.4011	SPOTW	21	Goleta	na	na	48.5	25.0	0.57
2286	117.3885	33.1667	SPOTW	22	Oceanside	95.0	98.7	1.8	22.5	0.25
2288	117.3844	33.1586	SPOTW	33	Oceanside	95.0	98.4	3.4	70.4	0.42
2273	119.1984	34.1305	SPOTW	14	Oxnard	56.7	nv	5.5	27.7	0.23
2274	119.1975	34.1238	SPOTW	16	Oxnard	na	na	2.4	6.0	0.14
2275	119.1955	34.1307	SPOTW	13	Oxnard	99.0	40.6	4.7	8.1	0.15
2276	119.1828	34.1237	SPOTW	14	Oxnard	na	na	5.8	7.0	0.15
2291	117.3526	33.0999	SPOTW	69	San Elijo	97.9	91.2	3.6	62.0	0.70
2293	117.2923	32.9995	SPOTW	23	San Elijo	92.5	86.2	1.0	0.8	1.82
2269	119.6674	34.3971	SPOTW	21	Santa Barbara	na	na	10.8	41.0	0.90
2272	119.6595	34.3982	SPOTW	23	Santa Barbara	na	na	11.7	23.0	0.44
2277	117.7649	33.5073	SPOTW	35	Terminal Island	98.0	100.0	6.8	56.7	0.73
2295	117.2987	32.9904	SPOTW	41	Terminal Island	97.8	100.0	3.8	36.7	0.41
2297	118.2353	33.7229	SPOTW	12	Terminal Island	88.3	93.2	10.4	78.4	1.40
2298	118.2340	33.7289	SPOTW	11	Terminal Island	87.2	100.0	8.7	69.8	0.93
2299	118.2340	33.7206	SPOTW	13	Terminal Island	91.4	87.4	12.3	65.2	1.36
2300	118.2391	33.7180	SPOTW	12	Terminal Island	95.8	100.0	3.6	86.1	1.34

<sup>a</sup> Strata Definitions:

Bath-30 = Offshore areas of shallow depth (5-30 m)

Bath-120 = Offshore areas of mid depth (30-120 m)

River = Sites near river mouths

River-gradient = Sites along a gradient of river influence

LPOTW = Areas near the outfall of a large publicly owned treatment works (POTW)

SPOTW = Areas near the outfall of a small publicly owned treatment works

Port = Port and industrial areas

Marina = Marina areas

Other = Harbor areas of mixed use

<sup>b</sup> Indicator Responses:

Amphipod = Percent of control survival

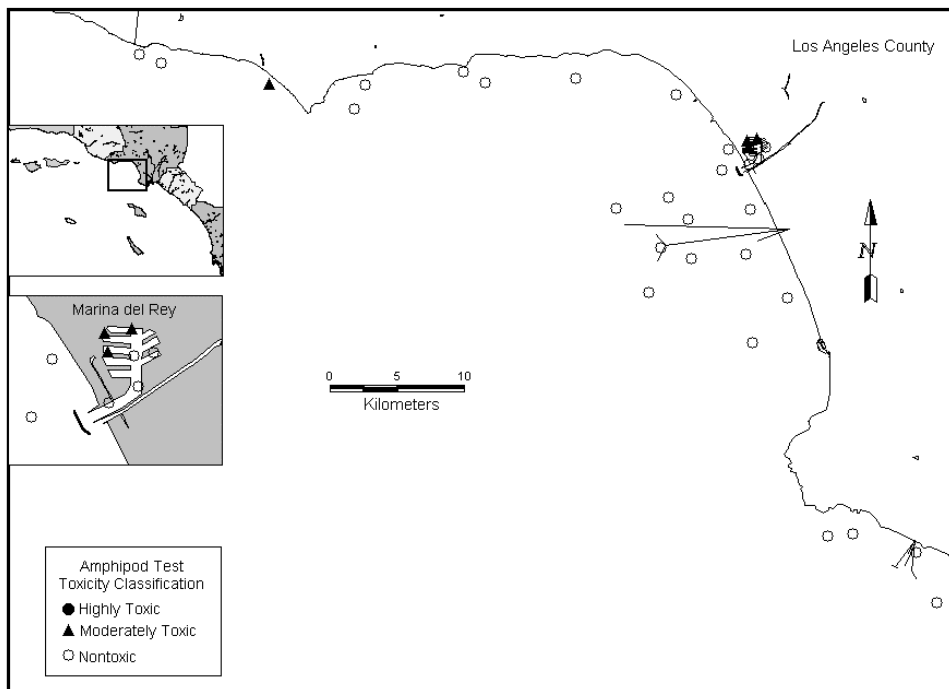
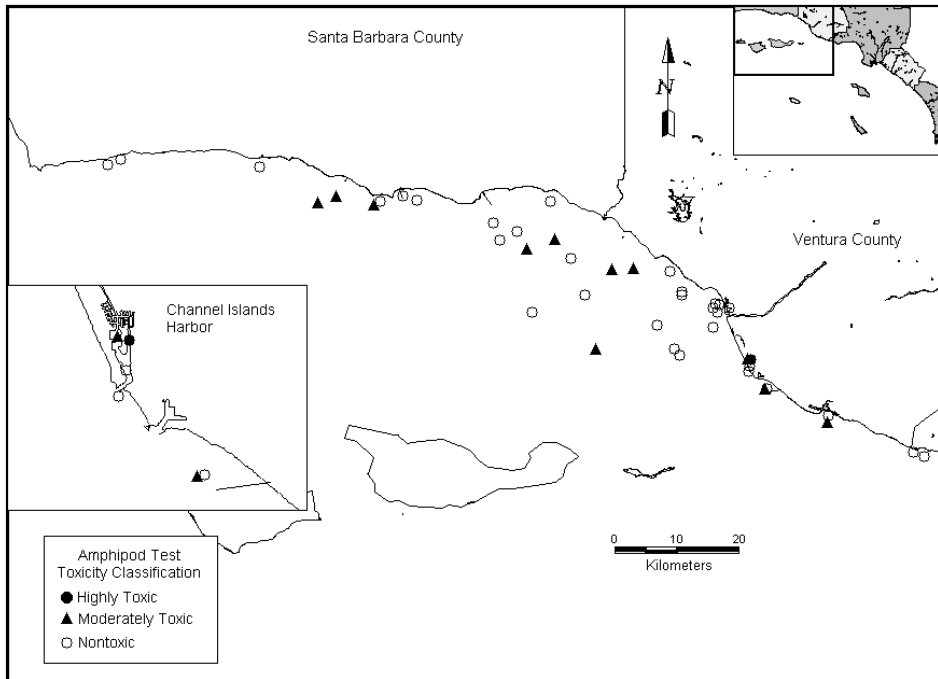
QwikSed = Percent of control luminescence

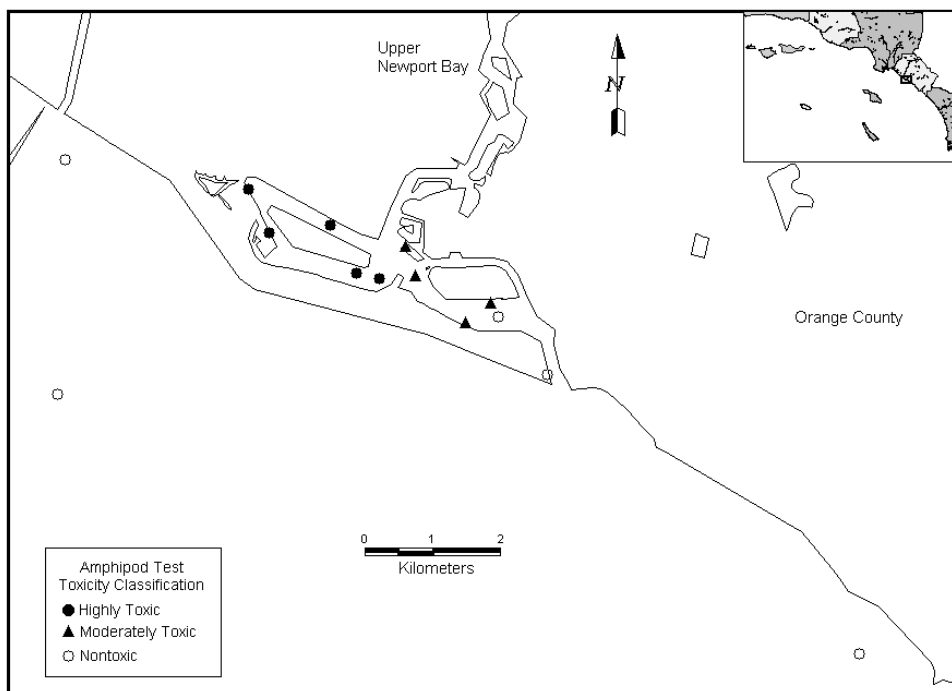
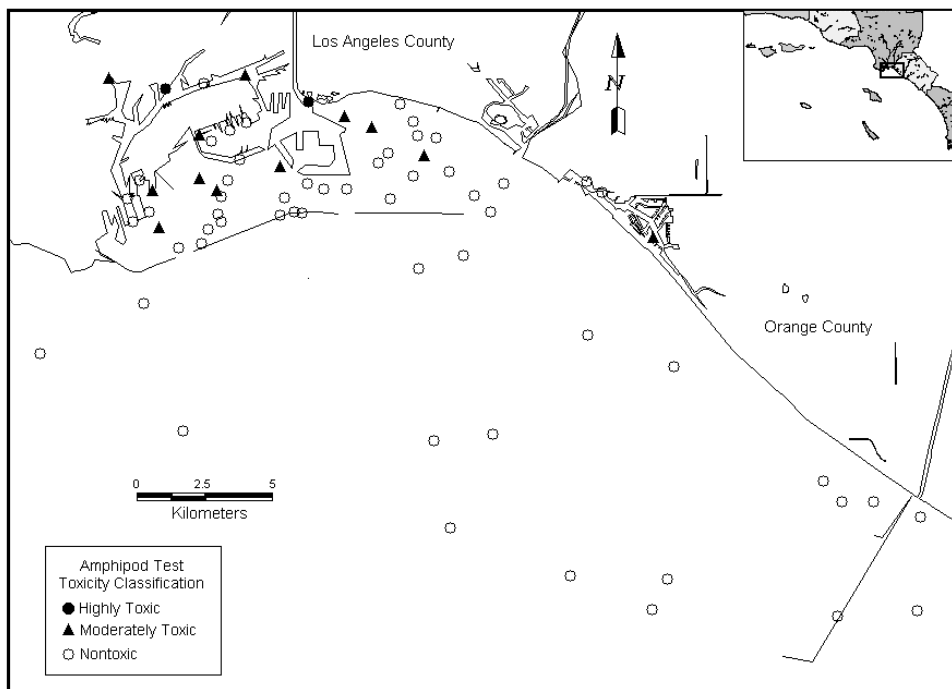
HRGS =  $\mu\text{g/g}$  benzo [a] pyrene equivalents

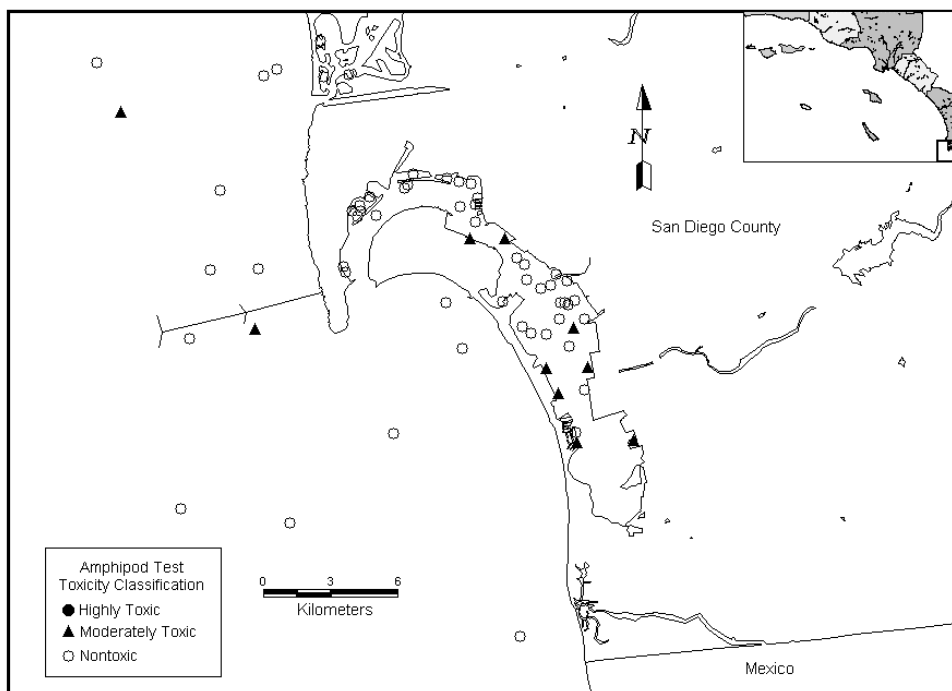
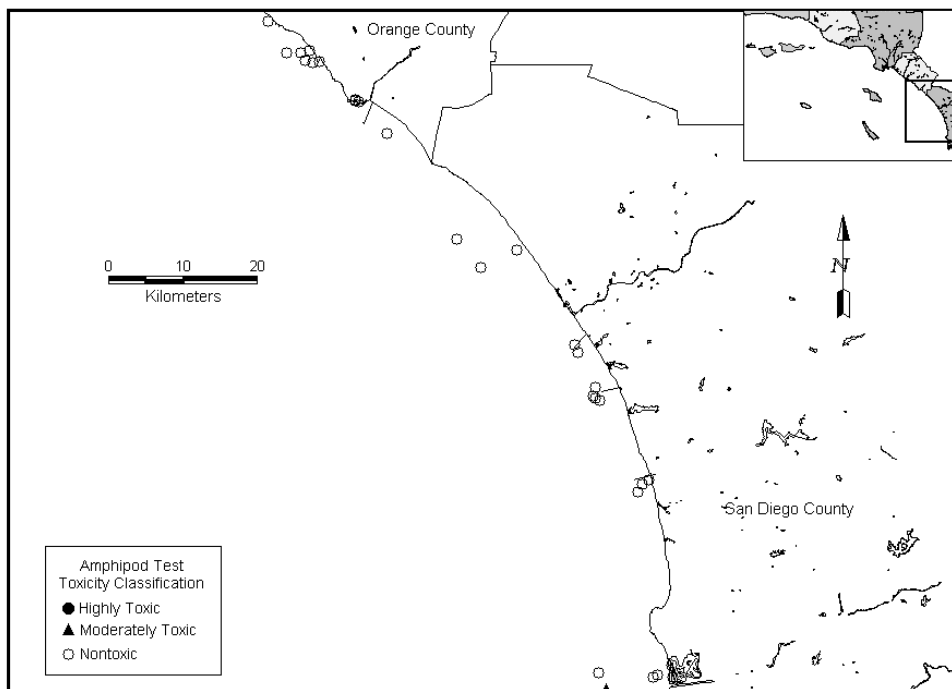
na = No sample was collected for the given analysis.

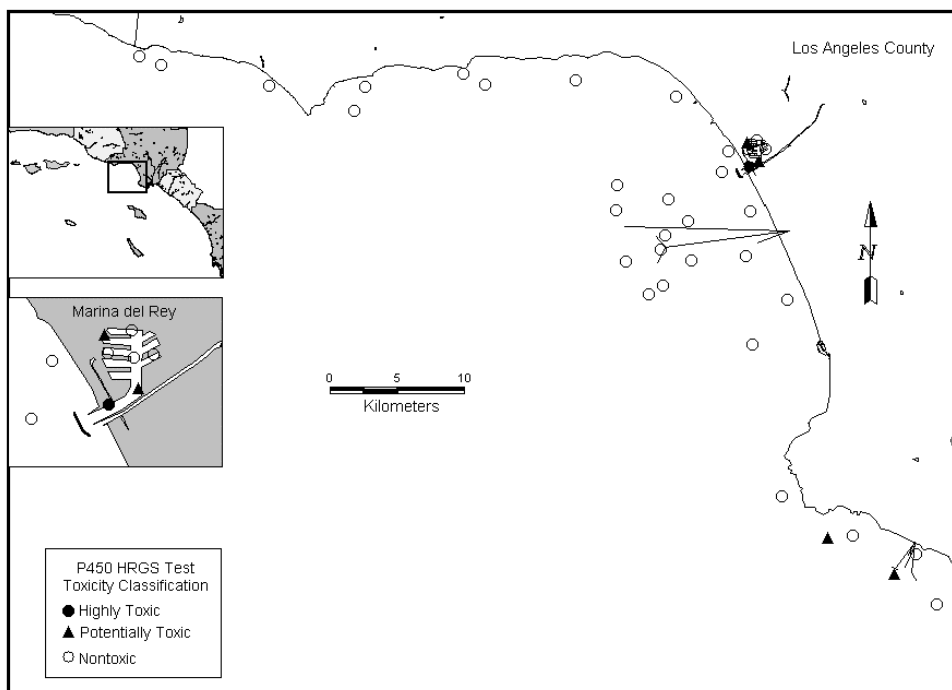
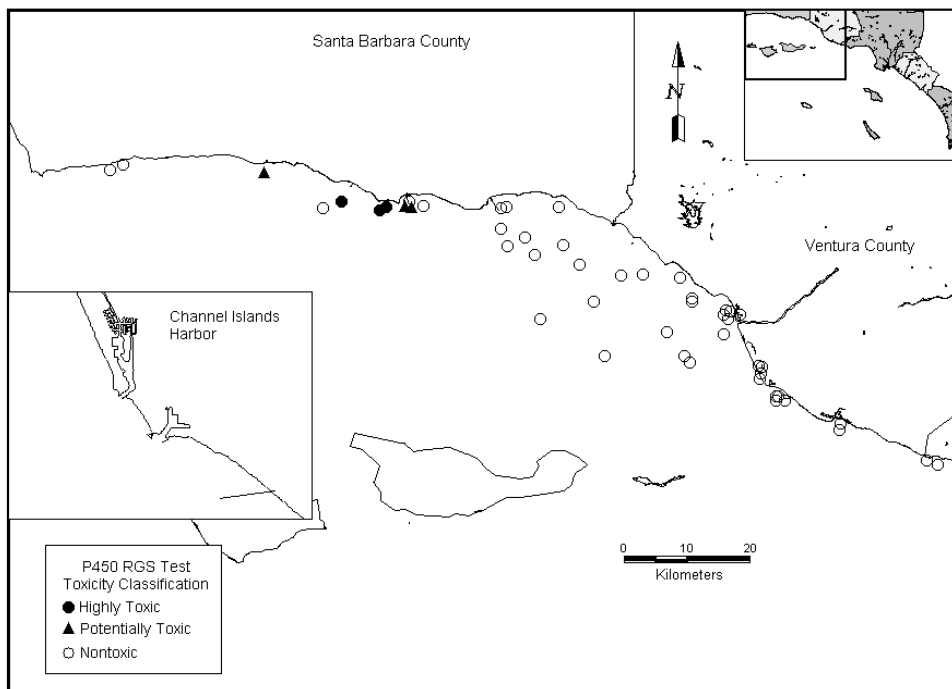
nv = No valid data were obtained from testing

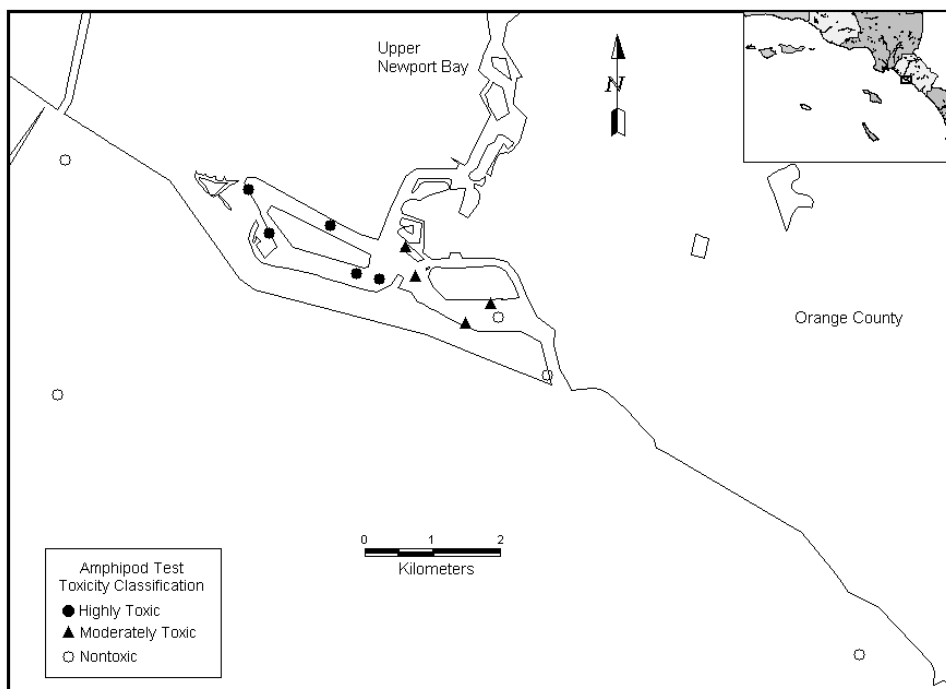
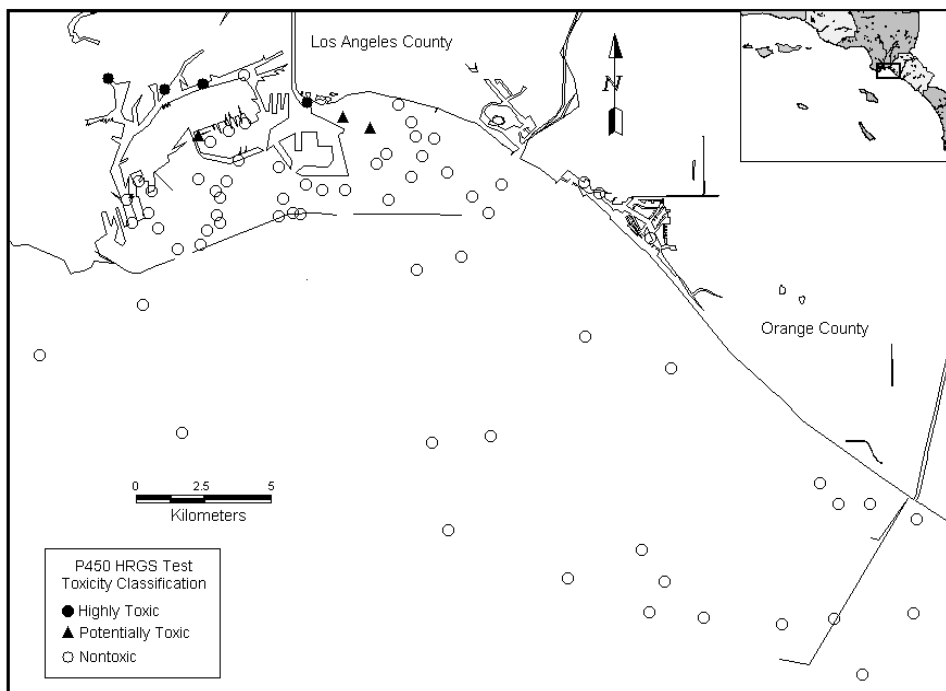
## APPENDIX E. TOXICITY TEST RESULTS MAPPED BY INDICATOR



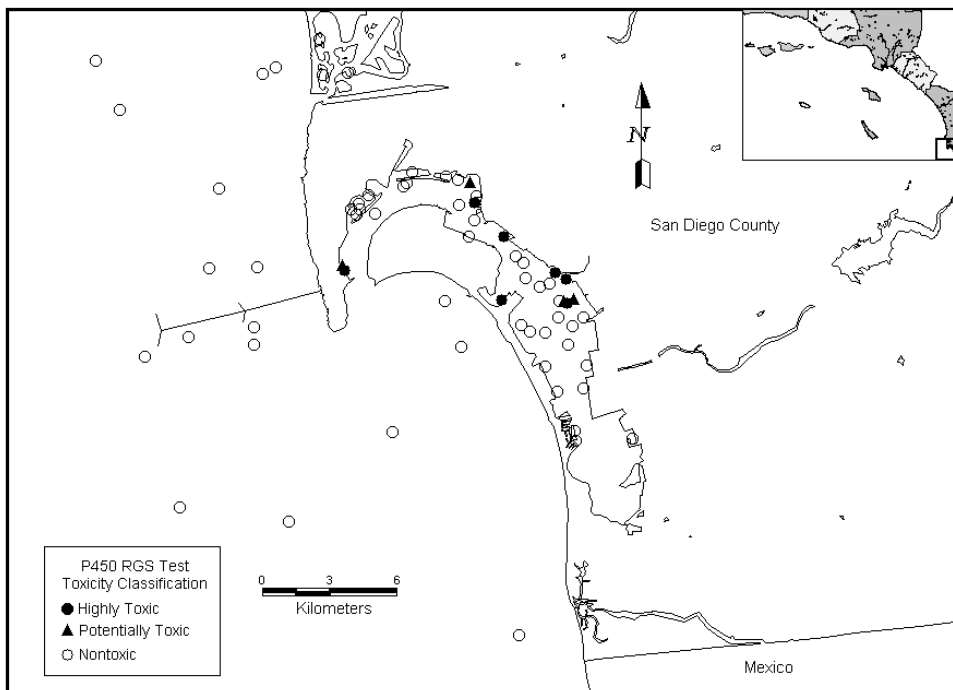
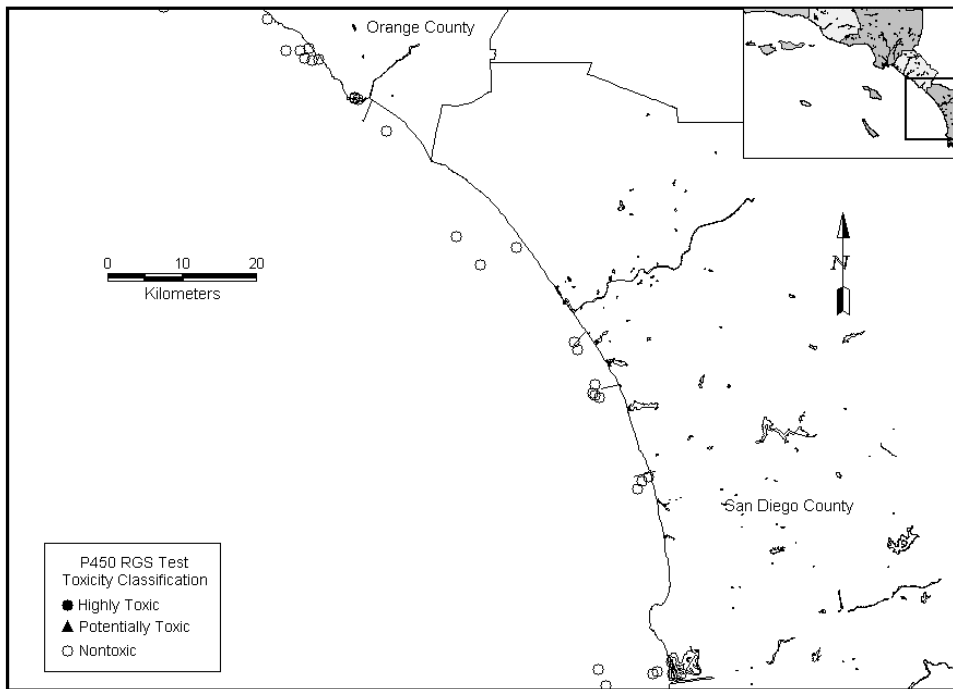


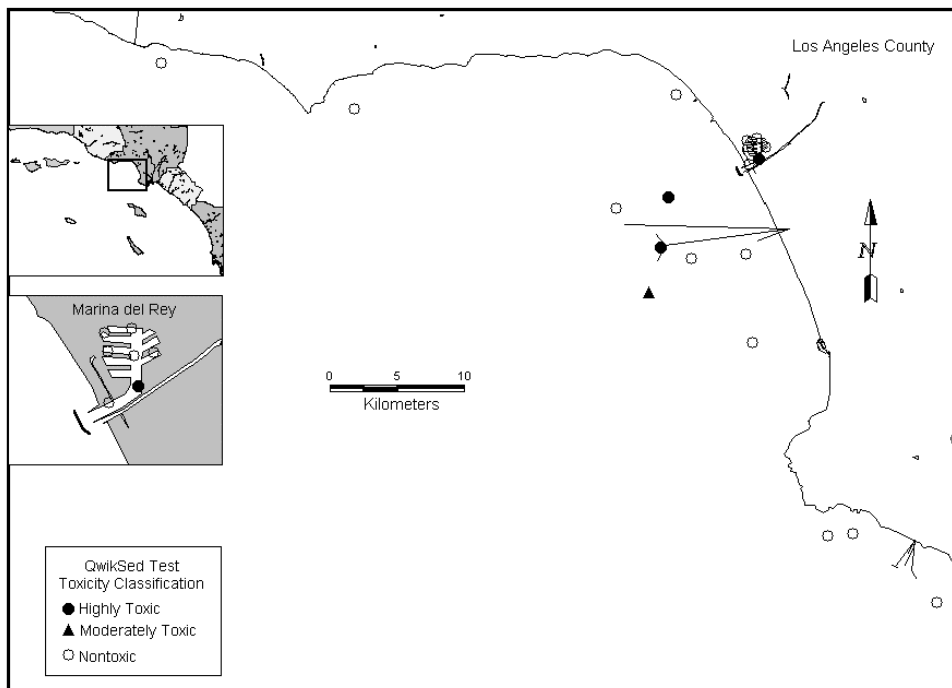
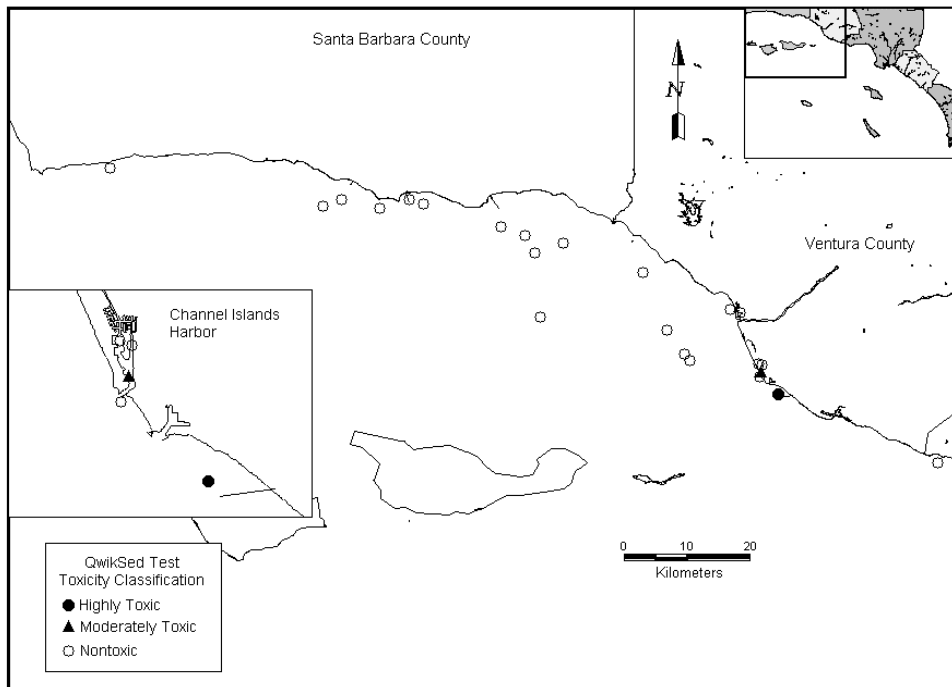


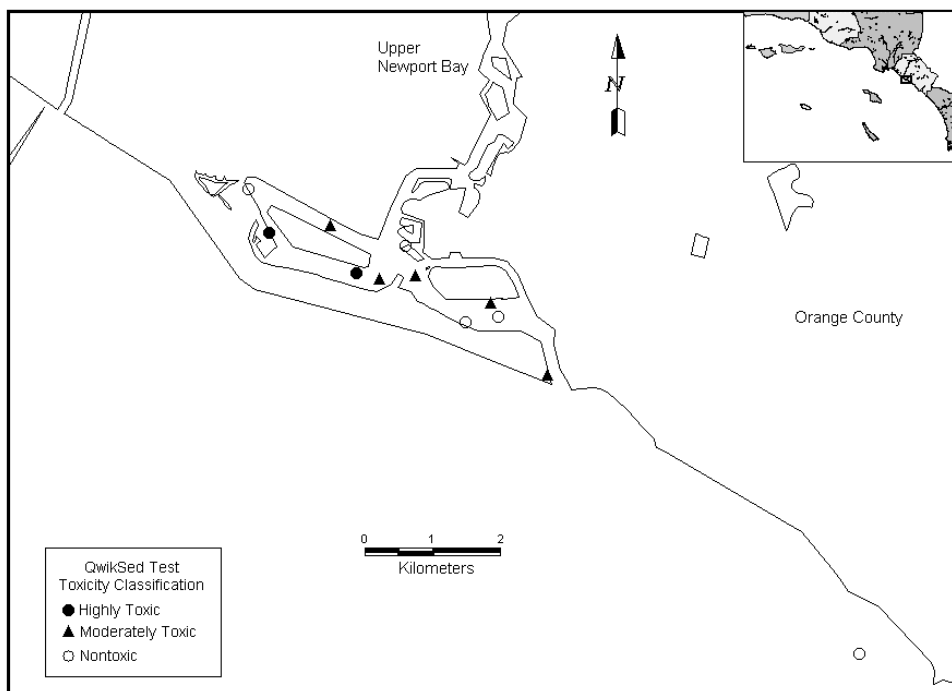
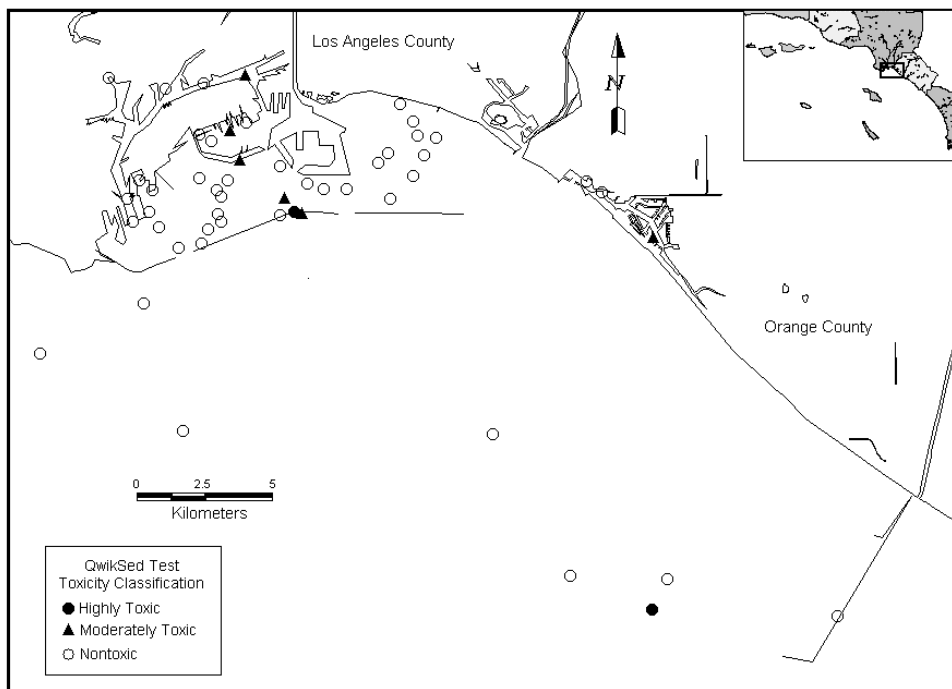


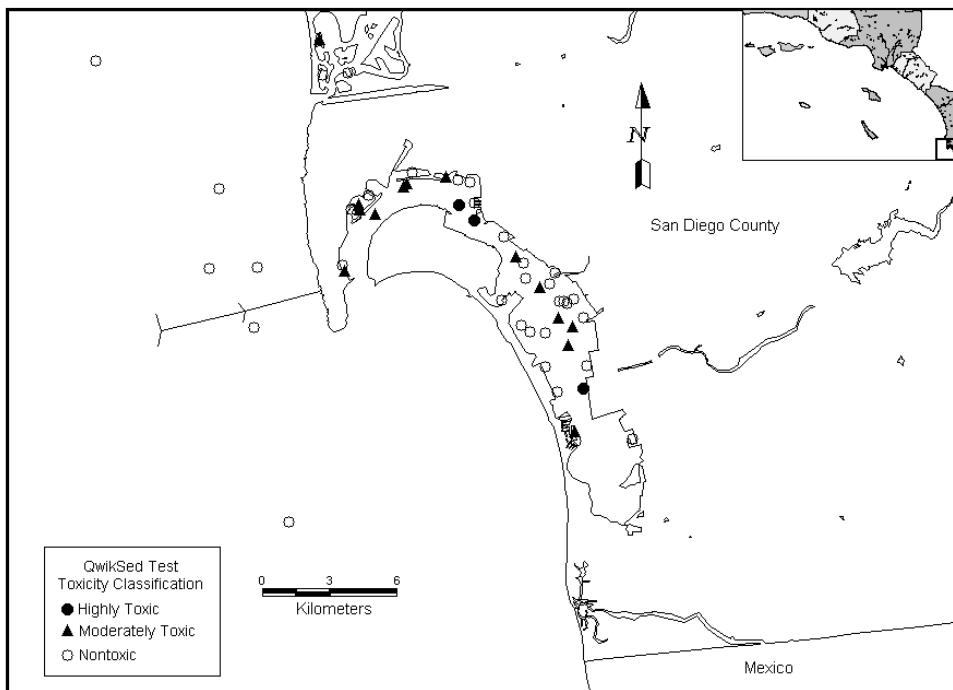
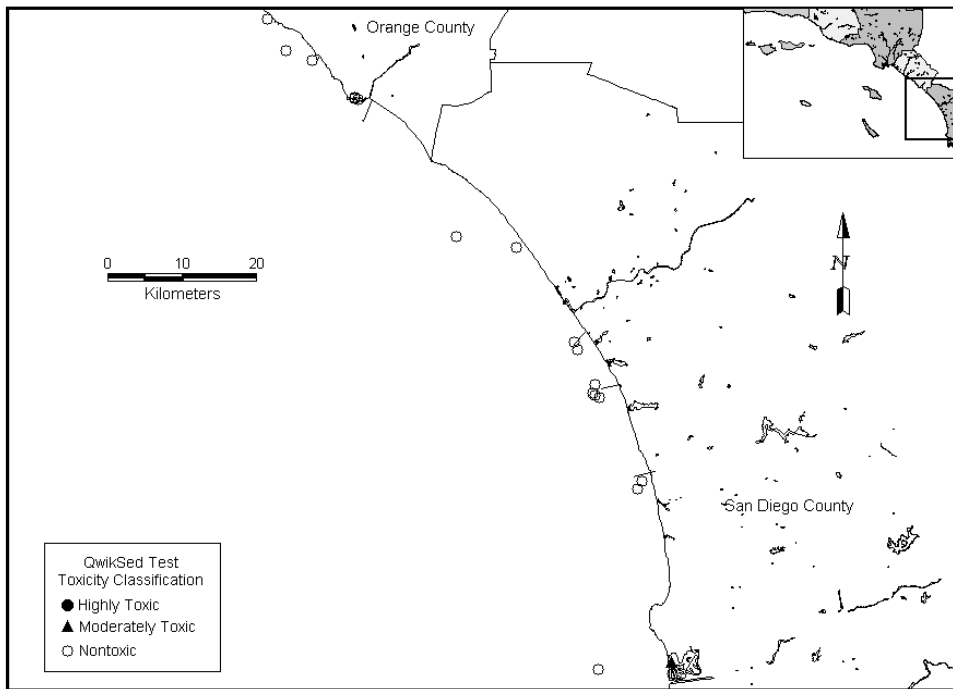












**APPENDIX F. RELATIONSHIP BETWEEN SEDIMENT CHARACTERISTICS  
AND INDICATOR RESPONSES**

