CHEMICAL ANALYSIS, TOXICITY EVALUATION AND BIOACCUMULATION EXPOSURE OF SEDIMENTS FROM HUMBOLDT BAY:

BASELINE SURVEY II

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FINAL REPORT

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BASELINE SURVEY II

1.0 Introduction

Under Contract No. DACW07-92-D-002 from San Francisco District, Army Corps of Engineers (SFACOE), ToxScan, Inc. collected and analyzed sediment samples from **Humboldt Bay** for FY 1994, **Baseline Survey II**. Sediments were sampled by Kinnetic Laboratories, Inc., and returned to the ToxScan, Inc. laboratory at Watsonville, CA where they were assigned laboratory number **T-10774** for physical, chemical and bioassay analyses. Bioaccumulation analyses were not performed on tissues exposed to these sediments. Samples collected, composites and analyses are summarized in Table 1.

2.0 Methods

2.1 Sediment Collection

Sediment sampling was conducted between 31 March and 5 April 1994 from the M/V Celtic and the M/V Sally Kae. Target sampling locations (California state plane coordinates) are listed in Table 1 of the Scope of Services provided by SFACOE (Appendix A, this report). The target locations were placed at or near sampling stations of Baseline Survey I (FY 1993) to establish an historical database of sediment characteristics at Humboldt Harbor. In some cases, sampling stations had to be moved from the target locations in order to sample areas with significant shoaling. Prior to initiating the field program each station's plane coordinates were converted to latitude/longitude coordinates to allow use of a differential Global Positioning System (GPS) for actual field positioning, and target locations were plotted on SFACOE-provided "blue line" pre-dredge survey charts to determine the site and approximate depth of each core. Actual sampling locations are plotted on Figures 1 through 4. Details of each core and grab sample (time collected, depth, location) are summarized in Table 2 and documented in field log sheets (Appendix B).

Horizontal positioning was established with a Trimble series 4000 Differential GPS navigation system with base station set on Corps of Engineers survey markers. Mudline elevations were determined at each core location at the time of sampling with a dual frequency fathometer calibrated by leadline to 0.1 feet at the expected depth range. Mean lower low water (MLLW) mudline elevations were extrapolated using Micronautics, Inc. Tide 1 software, and verified daily with USGS or COE benchmarks.

A preliminary field determination of sediment particle size distribution (PSD) was made at each sampling station, using a Smith-Macintyre grab sampler. Locations that yielded predominantly sand or coarser-grained material (80% $\Phi \leq 4$) were sampled for individual PSD analysis only. Stations that did not meet this criterion and showed greater than one foot of shoaling were sampled for discrete chemistry analysis using the Vibra-core sampler. Composite samples of material from the fine grained sites in each sampling area were made for chemistry, bioassay, and bioaccumulation analysis. Five composite samples were generated: 1) Eureka Upper Channel (EKUP); 2) Samoa Turning Basin (SAMTB); 3) Fields Landing Lower Channel and Turning Basin (FLTB); and 4) the disposal site reference (REF). Individual samples comprising each composite are indicated in Table 2. The composite samples were homogenized by thorough mixing using Teflon-lined containers and tools.

The vibracore cutting tip and core sample catcher were #306 grade stainless steel; the Vibracore barrel was aluminum. The Smith-Macintyre grab was constructed of galvanized steel. Prior to sampling at each station, the vibracore cutting tip, core catcher and the compositing equipment were all cleaned by the following EPA approved clean-up protocol (the Smith-Macintyre grab was cleaned with Steps 1 and 2 only):

1. Wash with 2% Micro Laboratory Soap
2. Rinse three times with clean water
3. Rinse with 2N nitric acid
4. Final rinse 3x with Milli-Q type I reagent grade DI water
5. Store in cleaned containers until use

2.1.1 Sample Handling. Vibracore and Smith Macintyre grab samples were taken during this project. Handling procedures for each sample type are summarized below:

Vibracore Samples. Each core sample was measured for total core length. If the core achieved penetration to project dredge depth the desired sample (from dredge depth to sediment surface) was extruded into the compositing container.

Grab Samples. Each grab sample was evaluated for grain size, composition, and penetration. Grabs which had "washed out", or which were determined to have insufficient penetration, were rejected.

The individual samples and area composites were placed in appropriate containers in precleaned coolers, on ice, to reduce the temperature to the prescribed 4°C. All samples were transported to ToxScan's chemistry and bioassay facilities in Watsonville under chain of custody at the prescribed temperature. Subsamples of the four composites were subsequently shipped at temperature under chain of custody to Alta Analytical Laboratory Inc., El Dorado Hills, CA for 2,3,7,8-TCDD and 2,3,7,8-TCDF (Dioxins) analysis.

2.2 Water Collection

Reference water for bioassay tests was collected at mid depth at the reference site using an EPA protocol-cleaned peristaltic pump and cleaned silicon and teflon hoses. The hose was lowered into the water at one end of the reference site, and the vessel drifted with the current through the reference site while sampling. The water was pumped into five (5) gallon cubitainers which were then stored at 4°C until delivery to the ToxScan laboratory in Watsonville.

2.3 Chemical and Physical Sediment Analysis

Sediment samples for chemical and physical analysis were collected in glass containers. Prior to analysis, samples were stored in the laboratory at 4°C. Analyses were conducted according to the following methods:

Sediment Grain Size was determined using the methods described in Plumb (1981).

Interstitial Water Salinity and Total Ammonia values were determined for centrifuge-extracted sediment pore waters by salinometer-calibrated refractometer (YSI Model 33 Conductivity/Salinity Meter and Atago S-10 or S-28 Hand Held Refractometer), and by pH meter / ammonia probe (Fisher Accumet Model 925 with Orion Ammonia Electrode Model 95-12). One hundred to two hundred grams of sediment were centrifuged at 7,000 to 8,000 rpm until supernatant was clear (15 - 30 minutes).

Total and Water Soluble Sulfides. This method was adapted from EPA Method 376.1 (EPA 1983) and Standard Method 4500-S^{-2}-E (APHA 1992). Sediment samples were mixed with O_2-free DIW, and treated in a manner similar to aqueous samples. Hydrogen sulfide present in aqueous samples was purged into a zinc acetate trap using nitrogen gas. The sample pH was adjusted to about 4 if total sulfide was to be determined, or left unadjusted for free sulfide determinations. The zinc sulfide precipitate in the trap was oxidized with a known and excess amount of iodine, and the unreacted iodine was back-titrated with thiosulfate.

Oil and Grease, Total Petroleum Hydrocarbon. Samples were acidified to a low pH and extracted with fluorocarbon-113 in a separatory funnel. The fluorocarbon layer was separated from each sample, passed over sodium sulfate and collected for analysis of Oil and Grease using an Infrared spectrophotometer scanning the wavelengths from 3200 to 2700 cm^{-1}. To determine Total Petroleum Hydrocarbons, this above extract was passed through silica gel which extracted the vegetable oil fractions; the remaining petroleum fraction was then analyzed by Infrared spectrophotometric techniques as described below.

Total Organic Carbon (TOC). Analysis for total organic carbon followed the method of Gaudette, et al. (1974). One-to-two grams of sediment were placed in a 500 ml flask to which 10 ml of potassium dichromate ($K_2CR_2O_7$) had been added. Twenty ml of concentrated sulfuric acid (H_2SO_4) was then added while the flask was swirled. After 30 minutes, the sample was diluted to a volume of 200 ml with de-

ionized water (DIW), and 10 ml of phosphoric acid (H_3PO_4) and 0.2 g of sodium fluoride (NaF) were added. After more swirling, 15 drops of diphenylamine indicator was added and the sample was titrated with 0.5N ferrous ammonium sulfate.

Metals. Analyses for metals employed combinations of the following Varian spectrophotometers: SpectrAA 400P or 400Z with GTA 96 a Graphite Furnace and autosampler; or a SpectrAA 10 with VOA 76 hydride—cold vapor generator and flame autosamplers. Sample preparation prior to analysis by atomic absorption was accomplished by guidelines specified by Chapter 3, Sections 3.2 and 3.3, 7000 series (EPA 1986).

Organotins. Organotin species analysis was by the method of Uhler and Durrel (1989). Speciation was done by a n-pentyl derivatization using a Gas Chromatograph with a Flame Photometric Detector. A sediment sample was mixed with 5 ml of hydrobromic acid (HBr), converting cationic butyltins to the bromide complexes, which were then extracted with a toluene-tropolone mixture. Following this extraction a n-pentylmagnesium bromide was used to convert the butyltins to the n-pentyl derivatives. This extract was cleaned by passing it through a Florisil/Silica chromatograph column and then injected into the Gas Chromatograph with a FPD detector where butyltins were quantified.

Chlorinated Pesticides and PCB's. Analyses for these constituents were determined by EPA Method 8080 (EPA 1986). Each solid sample was mixed with anhydrous sodium sulfate, placed in an extraction thimble and extracted using acetone and hexane in a Soxhlet extractor. The extract was then dried, concentrated, and, as necessary, underwent a Florisil clean-up. After extraction, a 2 microliter sample was injected into a gas chromatograph and the effluent detected by an electron capture detector.

Polynuclear Aromatic Hydrocarbons and Phthalates. Analyses for semivolatile compounds were by GC-MS techniques, following Method 8270 (EPA 1986). Each solid sample was mixed with anhydrous sodium sulfate, placed in an extraction thimble and extracted using acetone and hexane in a Soxhlet extractor. The extract was then dried, concentrated and cleaned up by gel permeation chromatography. After extraction, a 2 microliter sample was injected into a gas chromatograph and the effluent detected by mass spectroscopy.

TCDD and TCDF (Dioxins). Sediment samples were analyzed for 2,3,7,8-TCDD and 2,3,7,8-TCDF using EPA Method 8290. These analyses were performed by Alta Analytical Laboratory, Inc., El Dorado Hills, CA.

2.4 Bioassay and Bioaccumulation Test Procedures

2.4.1 Suspended Particulate Phase (SPP) Bioassays

Suspended particulate phase elutriates were prepared by procedures outlined in the "Green Book" (EPA/USACE 1991) using reference site water and test sediments. The test protocol for bivalves was as specified by ASTM (1989). Three concentrations (100%, 50%, 10%) of suspended particulate phase were tested. The lower concentrations were evaluated only if the 100% concentrations produced >50% inhibition of development. Three species were tested in suspended particulate phase bioassays: The larvae of a marine bivalve (the bay mussel, *Mytilus edulis*), a mysid (*Holmesimysis costata*), and a marine teleost fish (the speckled sanddab, *Citharichthys stigmaeous*).

Elutriate sanddab bioassays were performed at the Davenport laboratory, and elutriate bioassays with mysids and bivalve larvae were performed at the Watsonville laboratory. The positioning of test containers and other conditions in the laboratories were designed for uniform exposure to the controlled laboratory environment. Five replicates of test treatments were randomly assigned (complete random design) to the test containers by use of a random numbers generating program.

The sediment samples were placed in cleaned 5-gallon polyethylene buckets with laboratory seawater for elutriate preparation. The sediment to water ratio was 1:4 as specified in the Green Book. The mixtures were agitated by vigorous aeration for 30 minutes. After a one-hour settling period, the elutriates were siphoned off and used as suspended particulate phase media.

2.4.1.1 Bivalve Larvae (*Mytilus edulis*)

Mussels were induced to spawn by high-temperature stimulation. Eggs and sperm were collected in separate basins filled with aerated seawater at 20°C. Egg density was determined by microscopically counting several 1-ml aliquots taken from the well-mixed egg basin. Fertilization was accomplished by addition of an appropriate amount of sperm suspension, and confirmed by microscopic examination.

The control exposure, performed for quality assurance purposes, used seawater from our laboratory system. Five replicate dishes were used for each test exposure. Temperature, dissolved oxygen, pH and salinity were monitored in each test concentration and in controls at the beginning and end of the test.

Larvae were tested in 250 ml polyethylene beakers containing approximately 200 ml of test solution. After fertilization was confirmed an aliquot containing approximately 6000 fertilized eggs was pipetted into each test beaker. Gentle aeration was provided throughout the 48-hour duration of the test. Five extra beakers were prepared in addition to those required for test and control replicates. These "extra" test containers were not incubated for 48 hours, but rather they were evaluated immediately after

inoculation to provide the "initial recovery" data used to establish the mean number of embryos added to each experimental beaker.

At the end of the 48-hour exposure period the contents of each dish were poured through a 45μ nytex screen. Surviving larvae were retained on the screen. The test beaker was rinsed three times with seawater and each successive rinse was poured through the screen to ensure complete transfer of larvae. Larvae were quantitatively transferred from the screen into a graduated cylinder and the volume was adjusted with a seawater-formalin mixture. Contents of the cylinder were mixed by inversion to ensure uniform distribution of larvae, and a 1 ml aliquot was transferred to a Sedgwick-Rafter counting slide for microscopic evaluation. Larvae were scored for evidence of internal tissue inside a complete larval shell. Larvae which had a complete larval shell containing tissue were counted as normal, whereas empty shells and larvae with incomplete shells were scored as abnormal. Data were reported as percent of initial embryos which survived, and percent of survivors which showed normal development, as calculated below.

The raw data resulting from these bioassays included the following:

- Counts of embryos added to five replicate test containers which were <u>not</u> incubated for 48 hours (= initial recovery).

- Counts of normal and abnormal embryos from test containers (five replicates per sample, reference and control) which <u>were</u> incubated for 48 hours.

The results were calculated from these data as follows:

$$\%\ Survival = \frac{No.\ normal\ larvae\ recovered}{N} \times 100$$

$$\%\ Normal = \frac{No.\ normal\ larvae}{No.\ normal\ larvae + No.\ abnormal\ larvae} \times 100$$

where N = the mean initial number of embryos added (from initial recovery data).

For each test chamber other than controls, % survival data were adjusted to correct for mortality observed in the control exposures by use of **Abbott's correction**:

$$\textit{Corrected Sample \% Survival} = 100 - \left(\frac{\textit{mean \% control survival} - \textit{\% sample survival}}{\textit{mean \% control survival}} \times 100\right)$$

Percent normal development data were similarly adjusted.

For the bioassay to be considered a valid test, an average of at least 70% of the exposed embyros must survive in the controls; abnormals were counted as mortalities as per the Testing Guidelines contained in SFACOE Public Notice No. 93-2: Response to Comments on Public Notice 92-5.

Following the Scope of Services, the 100% elutriate concentrations were evaluated initially. If Abbott's-corrected survival or normal development values were $\geq$50%, no further evaluations were performed. If these values were $\leq$50%, the 10% and 50% elutriate exposures were evaluated and EC_{50} and/or LC_{50} calculations were made using the Trimmed Spearman-Karber method. For LC_{50} calculations, abnormal larvae and calculated mortalities were added; whereas for EC_{50} calculations, separate abnormality counts were used, as per Public Notice 93-2 (see above).

A reference toxicant bioassay was also performed for quality assurance purposes, to verify the health and sensitivity of the test organism population. The reference toxicant used was cupric sulfate ($CuSO_4 \cdot 5H_2O$) dissolved in laboratory seawater.

2.4.1.2 Mysid (*Holmesmysis costata*)

Adult mysids (*Holmesimysis costata*) were collected from kelp beds near Monterey, California. The animals were gently aggregated with a dip net, corralled into a submerged bucket without removing them from the water and transported directly to the bioassay lab. In transit, holding tank temperatures were maintained within 2°C of the ambient temperature at sampling. Gentle aeration was supplied from a bottle of compressed oxygen. Throughout testing, the mysids were fed about 50 brine shrimp (*Artemia salina*) nauplii per mysid per day to prevent mortality from starvation and cannibalism.

Mysids were tested in one-liter polycarbonate tanks containing one liter of test solution. To initiate testing, mysids were sorted into groups of 10 in small containers with very small volumes of seawater. Mysids were transferred to the test containers by submerging the containers and slowly tipping the animals into the test medium. During the bioassays, the number of survivors of the original 10 animals per tank were recorded as experimental data at 4, 8, 24, 48, 72, and 96 hours after test initiation. At each of these checkpoints, dead animals (i.e., those nonresponsive to mechanical stimulus) were removed from the test containers.

A reference toxicant bioassay was also performed on the mysids for quality assurance purposes, to verify the health and sensitivity of the test organism population. The reference toxicant used was Sodium Dodecyl Sulfate (SDS) dissolved in laboratory seawater.

2.4.1.3 Teleost Fish (*Citharichthys stigmaeus*)

Speckled sanddabs were collected by otter trawl from Tomales Bay and kept in holding tanks until transported to the laboratory via overnight delivery. They were allowed to acclimate to laboratory conditions prior to testing. Fish were fed a high protein pellet food during the holding period until 48 hours before test initiation; they were not fed thereafter.

Sanddabs were tested in 10-liter aquaria and were individually transferred from holding tanks to aquaria to start the test. During the bioassays, the number of survivors of the original 10 animals per tank was recorded as experimental data at 4, 8, 24, 48, 72, and 96 hours after test initiation. At each of these checkpoints, dead animals (i.e., those nonresponsive to mechanical stimulus) were removed from the test containers.

A reference toxicant bioassay was also performed on the sanddabs for quality assurance purposes, to verify the health and sensitivity of the test organism population. The reference toxicant used was Sodium Dodecyl Sulfate (SDS) dissolved in laboratory seawater.

2.4.1.4 Initial Mixing Calculations

In cases where an EC_{50} or LC_{50} was obtained, calculations of initial mixing were made using standardized formulae developed by the USACOE and EPA (EPA/ACOE 1977).

2.4.2 Solid Phase (SP) Static Bioassays (Amphipod)

Solid phase static bioassays were conducted on the harbor sediments simultaneously with control and reference sediments. The amphipod *Rhepoxynius abronius* was tested following procedures outlined in ASTM (1990).

Salinity and total ammonia measurements were made on sediment interstitial water as received; in addition, a final pore-water ammonia measurement was taken from one replicate of each test sediment at test termination. Pore waters were extracted by centrifugation. Interstitial water salinity was measured using a salinometer-calibrated refractometer. Interstitial water ammonia concentrations were measured with an ammonia probe calibrated to three concentration standards (see Sediment Physical and Chemical Analysis- Section 2.3).

In each test, five replicates of each station and reference treatment were randomly assigned to test jars. A 2-cm deep layer of appropriate sediment was added to each jar on the day prior to test initiation, and each test jar was provided with aeration via pasteur pipet. Each test was started on the following day by randomly assigning 20 amphipods to each jar, and continued for 10 days under static conditions with constant illumination and aeration. Daily measurements of environmental test conditions (temperature, salinity, pH, dissolved oxygen) were made in each test container, and the number of animals which had appeared on the sediment surface was noted.

At the end of the ten day exposure period, the contents of each jar were poured through a 0.5 mm sieve and the number of surviving amphipods counted. Survivors from each replicate were transferred into bowls containing control sediment and monitored for their ability to rebury within one hour. Test data for each replicate therefore include number of survivors and number of survivors able to rebury.

Reference toxicant bioassays were performed with each batch of test animals to verify the health and sensitivity of the test organism population. The reference toxicant used was cadmium chloride ($CdCl_2$) dissolved in laboratory seawater.

2.4.3 Solid Phase (SP) Flow-through Bioassays (Mysid Shrimp and Polychaete Worm)

Solid phase flow-through bioassays with mysids and worms were conducted on the harbor sediments simultaneously with control and reference sediments. Control sediments were collected from Tomales Bay. Testing for both species was performed at the Davenport facility where continuously flowing seawater is available, using testing procedures in EPA/COE (1991).

Mysids (*Holmesimysis costata*) were collected from kelp beds near Monterey, California. The animals were gently aggregated with a dip net, corralled into a submerged bucket without removing them from the water and transported directly to the bioassay lab. In transit, holding tank temperatures were maintained within 2°C of the ambient temperature at sampling. Gentle aeration was supplied from a bottle of compressed oxygen. Throughout testing, the mysids were fed about 50 brine shrimp (*Artemia salina*) nauplii per mysid per day to prevent mortality from starvation and cannibalism.

Polychaete worms (*Nephtys caecoides*) were collected from Tomales Bay and shipped overnight to the bioassay laboratory. They were kept in holding tanks with home sediment and overlying seawater until test initiation.

All sediments were sieved through a 1.0 mm screen to remove indigenous fauna, and a 3.0 cm layer of appropriate sediment was added to each test container. Tanks were then filled with lab seawater, and either twenty polychaete worms (*Nephtys caecoides*) or twenty mysids (*Holmesimysis costata*) were added to each container. Worms were tested in 31 L glass aquaria; mysids were tested in 1.5 L

polycarbonate tanks fitted with small, screened drain holes. The small mysid containers were suspended above the larger worm containers such that when the flow-through seawater system was activated, seawater passed through the mysid tanks, overflowed through the screened drain holes into the worm tanks, then drained to sea.

Solid Phase flow-through bioassays continued for 10 days. At least twice each day, environmental systems were checked for proper functioning. Once each day, the salinity and temperature of the system were measured. Dissolved oxygen and pH values of each tank were measured twice daily.

After the 10-day bioassay period, the contents of each tank were gently washed with seawater through a 0.5-mm nylon screen. The animals were retrieved from the screen and counted. Test data were the number of survivors of each species.

A reference toxicant bioassay was also performed on the mysids for quality assurance purposes, to verify the health and sensitivity of the test organism population. The reference toxicant used was Sodium Dodecyl Sulfate (SDS) dissolved in laboratory seawater.

2.4.4 Bioaccumulation Exposure

Based on evaluations of sediment chemistry, bioaccumulation tissue assessments were not performed on these sediments (see below). However, clams (*Macoma nasuta*) and polychaete worms (*Nephtys caecoides*) were exposed to test and control sediments in an array of 31-liter flow-through glass aquaria, as follows: Five replicates of each harbor composite, reference composite and control sediments were randomly assigned to the test tanks. The control sediment was collected from Tomales Bay, CA. Sediments were screened through a 1.0 mm screen to remove indigenous fauna, and a 3.0 cm layer was added to each tank. Tanks were filled with water and 30 clams and 40 worms were added to each. After a one-hour settling time, the flow-through seawater system was activated and adjusted to a flow rate equivalent to 5 tank/volume changes per 24 hours (6.5 liters/hour).

Bioaccumulation exposures continued for 28 days. At least twice each day, environmental systems were checked for proper function. Each tank was monitored daily for temperature and D.O., and the seawater system was monitored daily for salinity and pH.

After exposure, the contents of each tank were gently washed with seawater through a 0.5-mm nylon screen from which the animals were retrieved. Surviving clams were transferred for two days to filtered flowing seawater for gut evacuation. Surviving worms were transferred to 30-liter flow-through aquaria containing a 3-cm layer of fine, clean sand. Visual inspection of individuals confirmed how much time (typically 24 hours) was necessary for complete gut evacuation in worms. Directly following these treatments, the soft tissues of clams and worms were homogenized, then frozen.

3.0 Results

Sediment physical, chemical, and bioassay analyses are summarized in Table 1. Fourteen samples (including one replicate) from North Bay, Entrance and Bar were screened and analyzed only for particle size distribution (PSD). Twenty-seven samples were analyzed for PSD and sediment chemistry: 22 discrete samples plus four composites (EKUP, SAMTB, FLTB, REF) and the Tomales Bay control sediment. Bioassay testing and bioaccumulation exposures were performed on the four composites and on the control sediment; subsamples of these sediments were subcontracted for dioxin (2,3,7,8-TCDD and 2,3,7,8-TCDF) analysis.

3.1 Sediment Physical Analysis

The particle size distributions of the sediment samples and composites are summarized in Table 4 and detailed in Appendix C. Except for NB4, Rep 1, the North Bay, Entrance and Bar samples each contained at least 90% coarse sediments by weight ($\Phi \leq 4$). Coarse sediment composition of the three harbor composites were as follows: Eureka Upper Channel (EKUP) = 61.1%; Samoa Turning Basin (SAMTB) = 81.0%; and Field's Landing Lower Channel and Turning Basin (FLTB) = 42.6%. The disposal site reference (REF) composite contained 59.3% coarse sediments, and the Tomales Bay control sediment contained 97.9% coarse particles.

3.2 Bulk Sediment Chemistry

Results of bulk sediment chemical analyses of the Humboldt Harbor sediment samples and composites are summarized in Table 4. The laboratory reports are presented in Appendix C, and QA/QC reports are presented in Appendix D. Chains of Custody are Presented in Appendix F. The discussion below is generally limited to analyses of the harbor and reference composites; please refer to Appendix C for results of analyses of the individual samples.

Metals. The Humboldt Harbor sediment composites were analyzed for ten metals. Except for cadmium, metals concentrations in the Harbor composites were similar to or less than those found in the Reference composite. Within the Harbor composites, Comp FLTB tended to have the highest metals concentrations, and contained cadmium levels twice that of the reference composite. Individual accounts of the ten metals analyzed in these sediments are as follows:

- Arsenic concentrations ranged from 5.7 ppm to 7.0 ppm in the harbor composites. None of the harbor composites exceeded the 7.3 ppm found in the reference composite.

- Cadmium concentrations ranged from 0.1 ppm to 0.2 ppm in the harbor composites. FLTB and EKUP exceeded (by 2.0x) the 0.1 ppm found in the reference composite. It should be noted, however, that the cadmium concentrations found in these sediments were near the detection limit.

- Chromium concentrations ranged from 97 ppm to 100 ppm in the harbor composites. None of the harbor composites exceeded the 110 ppm found in the reference composite.

- Copper concentrations ranged from 12 ppm to 28 ppm in the harbor composites. Only FLTB (1.3x) exceeded the 21 ppm found in the reference composite.

- Lead concentrations ranged from 5.8 ppm to 8.1 ppm in the harbor composites. FLTB (1.09x) and EKUP (1.04x) exceeded the 7.4 ppm found in the reference composite.

- Mercury concentrations ranged from 0.08 ppm to 0.05 ppm in the harbor composites. None of the harbor samples exceeded the 0.20 ppm found in the reference composite.

- Nickel concentrations ranged from 62 ppm to 98 ppm in the harbor composites. Only FLTB (1.01x) exceeded the 97 ppm found in the reference composite.

- Selenium was not detected in any of the harbor composites, nor in the reference composite.

- Silver was not detected in any of the harbor composites, nor in the reference composite.

- Zinc concentrations ranged from 41 ppm to 66 ppm in the harbor composites. Only FLTB (1.06x) exceeded the 62 ppm found in the reference composite.

Butyltins. Three organotins (tri-, di-, and mono-butyltin) were measured in the Humboldt Harbor sediment composites. A small amount (1 ppb) of tributyltin was detected in the SAMTB composite. No mono- or tetrabutyltins were detected from the harbor composites, and the reference and control sediments contained no detectable butyltins.

Semivolatiles. Phthalate esters and seventeen polynuclear aromatic hydrocarbons (PAHs) were measured in the Humboldt Harbor sediment composites. Phthalate concentrations ranged from 160 to 170 ppb in the harbor composites; none exceeded the 1200 ppb measured in the reference composite. PAH detections were as follows:

LPAHs. Three low molecular weight PAHs were detected in the harbor or reference composites, as follows:

- 2-methylnaphthalene concentrations ranged from <20 ppb to 78 ppb in the harbor composites; FLTB (1.95x) and EKUP (1.03x) exceeded the 40 ppb detected in the reference composite.

- Naphthalene concentrations ranged from 37 ppb to 43 ppb in the harbor composites; all three harbor composites exceeded the <20 ppb detected in the reference composite.

- Phenanthrene concentrations ranged from <20 ppb to 49 ppb in the harbor composites; none of the harbor composites exceeded the 58 ppb detected in the reference composite.

HPAHs. Three high molecular weight PAHs (HPAHs) were detected in the harbor or reference composites, as follows:

- Fluoranthene concentrations ranged from 30 ppb to 42 ppb in the harbor composites; all three harbor composites exceeded the <20 ppb detected in the reference composite.
- Pyrene concentrations ranged from <20 ppb to 47 ppb in the harbor composites; FLTB and EKUP exceeded the <20 ppb detected in the reference composite.
- Benz(a)pyrene concentrations ranged from <20 ppb to 52 ppb in the harbor composites; only SAMTB exceeded the <20 ppb detected in the reference composite.

Chlorinated Pesticides and PCBs. The Humboldt Harbor sediment composites were analyzed for the eighteen chlorinated pesticides and four polychlorinated biphenyls (PCBs as Aroclors). None of the harbor composites, reference or Tomales Bay control sediments contained detectable amounts of these substances.

Dioxins. The Humboldt Harbor composites were analyzed for 3,7,8-TCDD and 3,7,8-TCDF by Alta Analytical Laboratories, (El Dorado Hills, CA). Only SAMTB (0.51 pg/g 2,3,7,8-TCDF) contained detectable quantities of dioxins.

Sediment Conventionals. Total sulfides ranged from 29 ppm to 110 ppm in the harbor sediment composites. The reference composite contained 0.3 ppm total sulfides. Except for a trace amount (0.2 ppm) in the FLTB composite, no water soluble sulfides were found in the harbor composites, nor in the reference composite.

Oil and Grease (31 ppm) was detected only in the FLTB composite; total petroleum hydrocarbons were not detected in the harbor and reference composites.

Percent solids in the harbor composites ranged from 65% to 77% compared to 73% in both the reference composite and the Tomales Bay control sediment; total organic carbon ranged from 0.3% to 0.8% in the harbor composites, compared to 0.4% in the reference composite.

Sediment Chemistry Summary: Except for total sulfides, Humboldt Harbor sediments appear to contain no particularly high concentrations of any of the tested substances or compounds when compared to the reference site sediments. Although sulfide concentrations in the harbor composites (29 ppm to 110 ppm) were not particularly high for harbor sediments in general, they exceeded the very low reference site concentration of 0.3 ppm. Concentrations of some PAHs (Naphthalene, Fluoranthene, Pyrene and Benzo(a)pyrene) in some harbor composites exceeded reference site values by 4.2x to 5.2x (calculating non-detects at 0.5 x D.L.), but again, the absolute concentrations (30 ppb to 52 ppb) of these compounds were not high.

3.3 Bioassay Test Results

Six sediment toxicity evaluations were conducted on the Humboldt Harbor sediments. Suspended particulate phase (SPP) bioassays and solid-phase (SP) bioassays employed a total of five species, and bioaccumulation exposures were conducted with two species (Table 3). Bioassay test results and statistical evaluations are summarized in Tables 6 through 11. Water quality monitoring data summaries and reference toxicant test results are tabularized in Appendix D. Logs of test animal shipping, receiving, acclimation and holding are contained in Appendix E.

3.3.1 Suspended Particulate Phase (SPP) Bioassays

Suspended Particulate Phase bioassay testing of the Humboldt Harbor Baseline Survey II sediments comprised three species: a bivalve larva (*Mytilus edulis*), a teleost fish (*Citharichthys stigmaeous*) and a mysid shrimp (*Holmesimysis costata*). Results of these bioassays are summarized below, and in Tables 6 through 8.

3.3.1.1 Bivalve Larvae

Adult *Mytilus edulis* were purchased from Carlsbad Aquafarm Inc., Carlsbad, CA. Collection data were not available. The animals were shipped on 13 April 1994 and arrived at ToxScan's Watsonville laboratory on 14 April via Federal Express overnight. The mussels were held in 33.2 - 33.9‰ seawater at 14.3 - 15.2 °C until test initiation on 20 April. Results of the bivalve larvae tests are presented in Table 6.

Survival. Mean survival of bivalves in the laboratory seawater control was 101.8%, well above the ASTM (1989) protocol requirements of 70 percent. The reference site 100% elutriate produced 86.4% survival, Abbott's-corrected to 84.9%. Abbott's corrected mean survival in the 100% elutriates of the Humboldt Harbor composites ranged from 76.6% in the FLTB composite to 93.2% in the SAMTB composite. None of the harbor sediment bivalve tests demonstrated enough toxicity to generate an LC_{50}.

Development. Mean normal development values (adjusted with Abbott's correction) for bivalve larvae exposed to 100% elutriates of the test sediment ranged from 88.3% in the FLTB composite to 99.7% in the EKUP composite. Normal development in the disposal site reference elutriate was 98.2%, Abbott's-corrected to 99.1%. Normal development the laboratory seawater control was 99.0%. None of the Humboldt Harbor sediment bivalve tests demonstrated enough toxicity to generate an EC_{50}.

Reference Toxicant. The bivalve reference toxicant LC_{50} was 5.72 ppb Cu (95% CL: 5.21 - 7.15), and the EC_{50} for development was 6.66 ppb (95% CL: 6.21 - 7.15). These values are within ±2 SD of the mean of EC_{50}s calculated from previous *Mytilus*: copper reference toxicant tests.