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From Commander, Western Division, Naval Facilities Engineering Command
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Subj: NAVAL STATION, TREASURE ISLAND, HUNTERS POINT ANNEX

Encl: (1) Draft Addendum, Environmental Sampling and Analysis Plan (ESAP)

1. In accordance with the Naval Station, Treasure Island, Hunters Point Annex Federal Facility Agreement, enclosure (1) is forwarded for your review and comment.
2. The ESAP can be implemented upon receipt by the Navy of Agency concurrence with enclosure (1). An expedited review and response will help ensure project implementation during the current rain season.
3. Should you have any questions regarding this matter, the point of contact is Commander, Western Division, Naval Facilities Engineering Command (Attn: Louise T. Lew, Code 1811, (415) 244-2552).

Original signed by:

MICHAEL A. MIGUEL
By direction

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ENVIRONMENTAL SAMPLING
AND ANALYSIS PLAN

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**ADDENDUM TO THE ENVIRONMENTAL SAMPLING
AND ANALYSIS PLAN FOR
NAVAL STATION, TREASURE ISLAND
HUNTERS POINT ANNEX
SAN FRANCISCO, CALIFORNIA**

October 27, 1991

Section 2.2.2 Selection of Control Station Area

On page 2-3, Section 2.2.2, the third bullet is modified as follows: "As a secondary criteria, the area containing sediments of similar physical characteristics as test (HPA) sediments (e.g. grain size). The emphasis on sediment physical characteristics will be on selecting a sediment that is matched primarily to the grain size preference of the organism and secondarily to the grain size of sediments at HPA."

On page 2-3, Section 2.2.2, reverse order of third and fourth bullets

On page 2-3, Section 2.2.2, a sentence is added to the end of the last paragraph which reads "If control sediment is purchased, the supplier will be required to furnish data to support the nontoxicity of the sediment".

Section 2.3.3.1 Amphipod Processing and Maintenance

On page 2-5, Section 2.3.3.1, the following sentence is added to the end of paragraph 2: "In order to initiate the amphipod bioassay tests, there will be no greater than 10% mortality in amphipods during the holding period prior to conducting the bioassays".

Section 2.4.1 Surficial Sediment Grab Sampling Procedures

On page 2-6, paragraph 1, the following sentence is added to the end of the paragraph: "All station locations will be reported in longitude and latitude".

On page 2-7, (paragraph 1), delete sentence one and two; replace with the following: "The ten grab sediment samples from random locations within each test station area (Plate 3) and one grab sediment sample from each reference station (Plate 6) will be obtained using a Van Veen grab sampler. The approximate volume of sediment per grab that will be collected by the Van Veen grab is 366 cubic inches".

On page 2-7, (paragraph 3), "A one-liter aliquot of sediment will be removed from the center of the grab at a consistent depth from the sediment water interface; the aliquot will be taken from the center of the grab in a manner to avoid contact with the sides. The sediment will be placed into a 15 liter container for mixing".

On page 2-7, paragraph 3, the second sentence is deleted. In Section 11.2.1.5, the EPA/COE Greenbook recommends using sieved sediments as soon as possible after the macroinvertebrates are removed. For this reason, as well as the reduced possibility of contamination of sediments during sieving procedures conducted in the laboratory as opposed to field sieving, sediment samples to be used in bioassay tests will be sieved in the laboratory. As press sieving for infauna will be conducted at the bioassay laboratory, sediment sieving is discussed in Section 2.6.1.1 and 2.6.1.2.

On page 2-7, delete paragraph 4 and replace with the following: "When the ten representative samples have all been transferred and the 15 liter container is filled, the sediment will be slowly stirred with a stainless steel rod to ensure adequate mixing. The sediment will be mixed until the color and texture are visually homogenized. Samples for physical and chemical analyses will be removed from the container and placed in the appropriate sample container, which will be filled to exclude air or overlying water, for the necessary subsamples. The 15 liter container, with the remaining portion of the composite sample, will be filled to overflowing with seawater in order to exclude air from the sample. The sample container will then be sealed and labeled with the station identification number for use in the bioassay tests. The 15 liter container will be stored immediately in an ice chest at 2° to 4° C and maintained at that temperature until the sediment is utilized in the bioassays. The amphipod sediment bioassay, modified solid-phase bioassay, and liquid suspended particulate phase bioassay will be initiated within fourteen days of sample collection".

Section 2.4.2 Sediment Core Sampling Procedures

On page 2-8, paragraph 1, the last sentence is changed to read "The location of each core sample station will be recorded using Loran C coordinates and will be reported in longitude and latitude".

On page 2-8, the last paragraph is modified to read "Sediment core samples will be collected from each station (Plate 3) using a 2-inch diameter gravity-type corer deployed from a boat. A minimum core penetration of three feet below the sediment-water interface will be achieved and a minimum of two feet of sediment will be collected from within the core.

On page 2-9, the second paragraph is modified to read "Discrete core samples will be extracted from the bottom 6 inches of the cores at the laboratory to avoid potential sample contamination in the field. The laboratory analytical program for sediment samples is discussed in Section 2.7 and summarized in Table 3."

Section 2.5 Preparation of Seawater for Bioassay Systems

On page 2-9, Section 2.5, paragraph 2 which discusses wet-sieving and static-renewal procedures is deleted.

Section 2.6.1.1 Sediment Preparation (Amphipod Sediment Bioassay)

On page 2-9, Section 2.6.1.1 the first bullet is modified as follows: "Sediments will be removed from the interior of the 10 liter composite sample".

On page 2-10, the first bullet is modified to read "Sediments will be pressed-sieved through a 0.5 mm mesh screen to remove infauna from the sediment".

On page 2-10, the following section will be deleted: "Prior to initiation of the bioassay, preparation of the test sediments will be conducted using the following methods:

- o Interstitial salinity of sediments will be determined by refractometer
- o Sediments will be placed in bioassay chambers with overlying water of a salinity calculated to raise interstitial sediments to a minimum of 15 ppt (if necessary)
- o Sediments will be slowly stirred by hand with a clean glass rod for one minute, then allowed to settle and equilibrate
- o Approximately 75% of the overlying water will be decanted and retained for use in the bioassay
- o Sediments will be mixed after reintroduction of the decant water to the test chambers
- o Interstitial salinities of each test chamber will be confirmed prior to initiation of the bioassay

Section 2.6.1.2 Test Chamber Systems

On page 2-10, the second paragraph of Section 2.6.1.2 starting with "Prepared seawater . . ." and continuing to page 2-11, is deleted.

Section 2.6.1.3 Introduction of Seawater and Sediments to Test Chambers

On page 2-11, Section 2.6.1.3, the third bullet is modified to read "Test chambers will be filled to the 950 mL mark on the test beaker with 15 ppt salinity seawater, covered with a watchglass and placed in a temperature controlled room. Sediment disturbance during seawater introduction will be minimized by placement of a disk on the sediment surface." This is the equivalent of adding approximately 775 mL of seawater to 175 mL of sediment which is a 4 to 1 water to sediment ratio.

Section 2.6.1.5 Initiation of Amphipod Sediment Bioassay

On page 2-12, the last bullet indicating that ammonia concentrations will be measured daily in the test chambers is deleted. The following sentence is added to the last paragraph in Section 2.6.1.5: "Ammonia concentrations in each test chambers will be measured and recorded at the initiation and completion of the bioassays."

Section 2.6.2.1

On page 2-12, the second bullet is modified as follows: "Sediments will be press sieved through a 0.5 mm mesh screen to remove infauna. Sediment will be retained in an uncontaminated container.

Section 2.6.2.3 Test Chamber Systems (Modified Solid-Phase Bioassays)

On page 2-13, Section 2.6.2.3, paragraph 2 is modified to read "Prepared seawater of approximately the same temperature, salinity and dissolved oxygen content as water from which the test organisms were collected will be used in the bioassay tests. Salinity will be maintained at ± 2 ‰ and temperature within ± 2 °C. Dissolved oxygen will be maintained above 40 percent saturation.

Section 2.6.2.4 Introduction of Seawater and Sediments to Test Chambers

On page 2-13, Section 2.6.2.4, the last bullet reading "Seventy-five percent of seawater volume in the test containers will be replaced using gentle siphoning and addition techniques one hour prior to addition of organisms." is deleted.

Section 2.6.2.6 Initiation of Bioassay

On page 2-14, Section 2.6.2.6, the last bullet reading "Ammonia concentrations in tank water" is deleted. A sentence is added to the following paragraph, that reads "Ammonia concentrations will be measured and recorded at the initiation and completion of the bioassays for each test chamber."

Section 2.6.4 Statistical Analysis and Interpretation of Results

On page 2-15, Section 2.6.4, the following sentences are added to the end of paragraph 1: "For solid-phase bioassays, the statistical hypotheses to be tested is that there is no difference in toxicity between the control sample and the test samples. The reference samples in South San Francisco bay will not be used in statistical comparisons against test samples from HPA to define toxic sediments".

Section 2.6.5 Liquid Suspended Particulate Phase Bioassays

Section 2.6.5, page 2-15 through 2-18, is deleted and is replaced with the following Sections 2.6.5, 2.6.5A, 2.6.5B, and 2.6.5C.

Section 2.6.5A Liquid Suspended Particulate Phase Bioassays - Mysid Shrimp

2.6.5.A.1 Test Organisms

Mysid shrimp, Holmesimysis costata, of similar size will be purchased for use in the mysid shrimp liquid suspended particulate phase bioassays. Organisms that are damaged during transport and handling will be discarded. Prior to initiation of bioassays, the mysid shrimp will be held in holding tanks with a minimum dissolved oxygen content of 60% saturation or greater. The mysid shrimp will be fed with a concentrated brine shrimp nauplii suspension twice a day during the holding period.

2.6.5.A.2 Organism Preparation

Just prior to initiation of the liquid suspended particulate phase bioassay, the following procedures will be conducted:

- o From holding tanks containing seawater, the mysid shrimp will be gently removed in fine-mesh nets.

- o Damage to the organisms will be avoided by handling with extreme care; mysid shrimp which appear damaged or that exhibit abnormal behavior will be discarded
- o Specimens of Holmesimysis costata of approximate equal size will be randomly divided into test containers so that each contains 10 individuals.

2.6.5.A.3 Sediment-Water Preparation

Prior to initiation of the liquid suspended particulate phase bioassay, preparation of the sediment-water mixture will be conducted using the following methods:

- o One liter sediment subsamples will be removed for each of the composite samples containers
- o The sediments will be combined with prepared artificial seawater in a volumetric sediment-to-water ratio of 1:4 at room temperature ($22^{\circ} \pm 2^{\circ}$ C)
- o The sediment-seawater mixture will be thoroughly mixed for 30 minutes
- o The mixture will then be allowed to settle for 1 hour
- o The liquid and sediment remaining in suspension after 1 hour will be siphoned off, taking care not to disturb the settled material, for immediate use in the bioassay.

2.6.5.A.4 Test Chamber System

Test chambers to be used in the mysid shrimp liquid suspended particulate phase bioassays will have a volume of 5 liters. Five replicate test containers will be used for the control station, the three reference stations and for each of the 17 test stations.

Prepared seawater of approximately the same temperature, salinity and dissolved oxygen content as the water from which the test organisms were collected will be used for the sediment-water mixture. Salinity will be maintained at ± 2 ‰ and temperature at $\pm 2^{\circ}$ C. A dissolved oxygen content of 40% saturation or greater will be maintained throughout the tests.

Three concentrations of test material suspensions will be tested at concentrations of 100, 50, and 10 percent.

2.6.5.A.5 Introduction of Seawater-Sediment Mixture to Test Chambers

The 1:4 sediment-water mixture will be introduced to the test chambers immediately upon completion of the sediment/water preparation procedures described in Section 2.6.5.3.

2.6.5.A.6 Introduction of Organisms to Test Chambers

Following preparation and selection of individual mysid shrimps for use in the bioassay, the organisms will be released to the chambers.

2.6.5.A.7 Initiation of Liquid Suspended Particulate Phase Bioassay

The bioassay will begin with the introduction of the mysid shrimp to the test tanks. The test duration will be 96 hours.

At 0, 4, 24, 48, 72, and 96 hours, the number of live mysid shrimp will be recorded. An organism will be considered dead if it does not respond to the probing of a sensitive body part and will be removed from the test chamber. In addition, any behavioral abnormalities exhibited by test organisms will be recorded. At each observation period, dead organisms, molted exoskeletons, and food debris will be removed from the test chambers by pipette or forceps.

Daily levels of the following water parameters will be measured and recorded:

- o Salinity of test water
- o Temperature of test water
- o Dissolved oxygen content of test water
- o pH of test water.

The tank water will be aerated only when necessary to maintain the dissolved oxygen content above 40% saturation.

2.6.5.A.8 Completion of Bioassay

After 96 hours, the tank water containing the mysid shrimp will be searched thoroughly for organisms. The organisms will be considered alive if they show

any response to the gentle probing of sensitive parts or gently swirling of the water. The number of live organisms will be counted and recorded.

2.6.5.A.9 Presentation of Data

If control mortality is greater than 10 percent, the results of the bioassay may not be evaluated. In this case, species selection and other test variables will be reevaluated in an attempt to reduce unacceptably high control mortality. If control mortality is less than 10 percent, the bioassay data will be presented in tabular form and will contain the following information:

- o Scientific name of test species
- o Number of organisms in each treatment at test start
- o Number of organisms alive at each observation period
- o Number of organisms recovered alive at test end
- o Any behavioral abnormalities recorded.

2.6.5.A.10 Statistical Analysis and Interpretation of Results

If control mortality is less than 10 percent and is less than the mortality in the test material treatment, the test data will be statistically analyzed to determine if there is a significant difference in survival between control and test samples. The t-test (Snedecor and Cochran, 1980) will be used to compare the mean control and test survivals following the Levine's test for the homogeneity of sample variances.

If mortality in the test material exceeds 50 percent, an LC50 value (lethal concentration to 50 percent of the test organisms) will be calculated for any dilutions in which greater than 50 percent mortality occurs.

In the event that no mortality occurs in either control or test tanks, or that survival of organisms in the test tanks is equal to or greater than control organism survival, no statistical analyses will be performed.

2.6.5.B Liquid Suspended Particulate Bioassay - Bivalve Larvae

2.6.5.B.1 Test Organisms

Upon purchase, adults (*Crassostrea gigas* or *Mytilus edulis*) will be transported without delay to the laboratory, cleaned of detritus and fouling organisms

such as barnacles, and placed in flowing water with a salinity and temperature suitable to the species ($\pm 2\text{‰}$ and $\pm 2^{\circ}\text{C}$ that of the collection or culture water). Adults that are injured during handling will be discarded.

The brood stock will be gradually conditioned to the test temperature and salinity. Temperature will be changed at a rate not to exceed $2^{\circ}\text{C}/\text{day}$ and salinity at a rate not to exceed $5\text{ g/kg}/\text{day}$. The concentration of dissolved oxygen will be maintained between 60 to 100% of saturation. The adults will be provided with cultivated phytoplankton to prevent malnutrition during the conditioning and holding period. The brood stock will be carefully observed daily during holding and conditioning for signs of stress and mortality. Dead bivalves and gaping mollusks that do not close when touched with a probe will be discarded daily.

Embryos used in the bioassay will be obtained from females and males that have been maintained for at least two weeks in the dilution water in the laboratory before they are induced to spawn.

2.6.5.B.2 Organism Preparation

Prior to initiation of the bivalve larvae bioassay, the following procedures will be conducted:

- o In preparation for thermal stimulation of spawning, 10 to 50 animals will be selected from a population of bivalves with ripe gonads and placed in small groups in spawning chambers (Pyrex dishes)
- o Chambers will be filled with dilution water at the conditioning temperature, and the animals will be allowed to begin pumping before starting thermal stimulation
- o The spawning chambers will be placed in a water bath filled with hot water
- o When the temperature in the spawning chamber attains a temperature of 5 to 10°C above the conditioning temperature, the water bath will be drained
- o Females will be additionally stimulated to spawn by the addition of sperm from a sacrificed or naturally spawned male
- o The spawning animal will be left in the chamber until the release of gametes ceases, at which time it will be returned to a holding tank

- o Eggs will be passed through a 75 um screen and the concentration of eggs determined by counting a sample of the egg suspension. The egg density will be adjusted to the range 20 to 50 eggs/mL before adding sperm
- o After sperm have been verified by microscopic examination, the sperm suspension will be passed through a 37-um screen to remove feces and other extraneous material
- o Within one hour of spawning, eggs and sperm will be combined in a one liter Nalgene beaker
- o Fertilization will be accomplished at the spawning temperature and the suspension held at that temperature until it is determined that fertilization has been accomplished
- o After the eggs have been fertilized, the embryo suspension will be poured through a 54-um screen to remove debris. Excess sperm, small protozoa, and bacteria will be removed by pouring the embryos onto a 22-um screen, washing with dilution water and backwashing into a suitable container filled with filtered seawater at incubating temperature
- o Embryos will be agitated using a perforated plunger to keep them in suspension and will be utilized in the bioassay within 2 hours of fertilization.

2.6.5.B.3 Sediment-Water Preparation

Prior to initiation of the liquid suspended particulate phase bioassay, preparation of the sediment-water mixture will be conducted using the following methods:

- o One liter sediment subsamples will be removed for each of the composite sample containers
- o The sediments will be combined with prepared artificial seawater in a volumetric sediment-to-water ration of 1:4 at room temperature
- o The sediment-seawater mixture will be thoroughly mixed for 30 minutes
- o The mixture will then be allowed to settle for 1 hour

- o The liquid and sediment remaining in suspension after 1 hour will be siphoned off, taking care not to disturb the settled material, for immediate use in the bioassay
- o The temperature of the test media will be adjusted to the appropriate temperature for the organism embryo tested (C. gigas - 20^o C, M. edulis - 15^o C).

2.6.5.B.4 Test Chamber System

Test containers to be used in the liquid suspended particulate phase bioassays for the bivalve larvae will have a volume of 1 liter. Five replicate test containers will be used for the control station, the three reference stations and for each of the 17 test stations.

Prepared seawater of approximately the same temperature, salinity and dissolved oxygen content as the water from which the test organisms were collected will be used for the sediment-water mixture. Salinity will be maintained at ± 2 ‰ and temperature at ± 2 °C.

Three concentrations of test material suspension will be tested at concentration of 100, 50, and 10 percent.

2.6.5.B.5 Introduction of Seawater-Sediment Mixture to Test Chambers

The 1:4 sediment-water mixture will be introduced to the test chambers immediately upon completion of the sediment/water preparation procedures.

2.6.5.B.6 Introduction of Organisms to Test Chambers

About 1 hour after adding the sperm suspension to the egg suspension, the concentration of embryos in the embryo suspension will be determined by mixing the solution with a perforate plunger, withdrawing a 1-mL sample, placing it in a Sedgwick-Rafter cell, and counting the number of embryos that have developed to the 2-cell stage or beyond. The concentration of embryos in the test solution will be between 15 and 30 embryos per mL.

Concentrations of up to 100 embryos per milliliter do not impair normal development of Crassostrea gigas. Mytilus edulis will develop abnormally at concentrations above 30 embryos/mL.

Within 4 hours after fertilization, equal volumes of the homogeneously mixed embryo suspension will be placed in each test container, which already contains the test solution, in a random order by using a pipet.

2.6.5.B.7 Initiation of Liquid Suspended Particulate Phase Bioassay

The bioassay will begin with the introduction of organisms to the test containers. The test duration will be 48 hours. The organisms will not be fed during the test as uneaten food might decrease the dissolved oxygen content and the biological activity of some test materials.

Levels of the following water parameters will be measured at the beginning and end of each test and recorded:

- o Salinity of the test container water
- o pH of the test container water
- o Dissolved oxygen content of the test container water
- o Daily maximum and minimum temperature of the test container water.

The test container water will not be aerated during the test as bubbles can collect within the mantle cavity of the larvae, and adversely affect larval survival and development.

2.6.5.B.8 Completion of Bioassay

Forty eight hours after beginning the test, the solution in each test chamber will be carefully mixed and preserved in a 5 percent buffered formalin solution. The embryos and larvae will then be placed in a Sedgwick-Rafter counting chamber.

All embryos exhibiting cell division will be counted. Percent abnormality will be determined by enumerating normal and abnormal larvae. All larvae with completely developed shells containing meat will be counted as normal. Empty shells, even if they are completely developed, will not be counted as the larvae are not alive at the end of the test. Larvae with incompletely developed shells after 48 hours may be morphologically normal, but the retarded development is considered likely to reduce their survival in the natural environment. Percent survival will be determined as the number of larvae surviving in each test container relative to the seawater control.

2.6.5.B.9 Presentation of Data

If control mortality is greater than 20 percent, the results of the bioassay may not be evaluated. In this case, species selection and other test variables will be reevaluated in an attempt to reduce unacceptably high control mortality.

If control mortality is less than 20 percent, the bioassay data will be presented in tabular form and will contain the following information:

- o Scientific name of test species
- o Water quality data measured at the beginning and end of testing (salinity, temperature, dissolved oxygen content, and pH)
- o Individual replicate, and mean and standard deviation data for larval survival after 48 hours
- o Individual replicate, and mean and standard deviation data for larval abnormalities after 48 hours
- o 48 hour LC50 and EC 50 values with reference toxicants.

2.5.6.B.10 Statistical Analysis and Interpretation of Results

If control mortality is less than 20 percent and is less than the mortality in the test material treatment, the test data will be statistically analyzed to determine if there is a significant difference in survival between control and test samples. The t-test (Snedecor and Cochran, 1980) will be used to compare the mean control and test survivals following the Levine's test for the homogeneity of sample variances.

LC50 value (lethal concentration to 50 percent of the test animals) will be calculated for any dilutions in which greater than 50 percent mortality occurs.

In the event that no mortality occurs in either control or test tanks, or that survival of organisms in the test containers is equal to or greater than control organism survival, no statistical analyses will be performed.

2.6.5.C Liquid Suspended Particulate Phase Bioassay - Sanddab

2.6.5.C.1 Test Organisms

Juvenile sanddabs (Citharichthys stigmaeus) less than 90 days old and of similar size will be purchased from a commercial supplier of wild collected animals for use in the liquid suspended particulate phase bioassay. The organisms will be inspected to assure that they are in good condition and free from disease. Fish that are injured during handling or that appear diseased will be discarded. To assure healthy test organisms, the fish will be held in the laboratory for a minimum two-day acclimation period.

Loading of fish in holding tanks will not exceed one g/L to avoid overcrowding. The dissolved oxygen content will be maintained by aeration at a minimum of 40% saturation during holding and acclimation periods. Fish will be fed adlibitum at least once a day with brine shrimp during the holding period up to two days before the bioassay test begins. Excess food and fecal material will be removed from the bottom of the tanks. Organisms will be observed carefully each day for signs of disease, stress, physical damage, and mortality. Dead and abnormal specimens will be removed as soon as observed. If greater than 10 percent of the total population becomes infected, the holding tank population will be destroyed.

If the water in which the fish are received differs from test water conditions, they will be gradually conditioned to the test temperature and salinity. Temperature will be changed at a rate not to exceed 2^o C/day and salinity at a rate not to exceed 5 g/kg/day.

2.6.5.C.2 Organism Preparation

Just prior to initiation of the liquid suspended particulate phase bioassay, the following procedures will be conducted:

- o From holding tanks containing seawater, the fish will be gently removed and transferred in fine-mesh nets
- o Damage to the organisms will be avoided by handling with extreme care; organisms which appear damaged or that exhibit abnormal behavior will be discarded
- o Juvenile sanddabs of approximate equal size will be randomly divided into test containers so that each contains 10 individuals.

2.6.5.C.3 Sediment-Water Preparation

Prior to initiation of the liquid suspended particulate phase bioassay, preparation of the sediment-water mixture will be conducted using the following methods:

- o One liter sediment subsamples will be removed for each of the composite sample containers
- o The sediments will be combined with prepared artificial seawater in a volumetric sediment-to-water ratio of 1:4 at room temperature (22^o ± 2^o C)

- o The sediment-seawater mixture will be thoroughly mixed for 30 minutes
- o The mixture will then be allowed to settle for 1 hour
- o The liquid and sediment remaining in suspension after 1 hour will be siphoned off, taking care not to disturb the settled material, for immediate use in the bioassay.
- o The temperature of the test media will be adjusted to the appropriate test temperature ($20^{\circ} \pm 1^{\circ} \text{C}$)

2.6.5.C.4 Test Chamber System

Tanks to be used in the sanddab liquid suspended particulate phase bioassays will have a volume of at least 10 liters. Five replicate tanks will be used for the control station, the three reference stations and for each of the 17 test stations.

Prepared seawater of approximately the same temperature, salinity and dissolved oxygen content as the water from which the test organisms were collected will be used for the sediment-water mixture. Salinity will be maintained at ± 2 ‰ and temperature at $\pm 2^{\circ} \text{C}$. A dissolved oxygen content of 40 percent or greater will be maintained throughout the tests.

Three concentrations of test material suspension will be tested at concentrations of 100, 50, and 10 percent.

2.6.5.C.5 Introduction of Seawater-Sediment Mixture to Test Tanks

The 1:4 sediment-water mixture will be introduced to the test tanks immediately upon completion of the sediment/water preparation procedures described in Section 2.6.5.3B.

2.6.5.C.6 Introduction of Organisms to Test Tanks

Following preparation and selection of individual fish for use in the bioassay, the fish will be released to the tanks.

2.6.5.C.7 Initiation of Liquid Suspended Particulate Phase Bioassay

The bioassay will begin with the introduction of the fish to the test tanks. The test duration for the sanddab will be 96 hours.

At 0, 4, 24, 72 and 96 hours, the number of live fish will be recorded. A fish will be considered dead if it does not respond to prodding and will be removed from the test tank. In addition, any behavioral abnormalities exhibited by test organisms will be recorded. At each observation period, dead fish will be removed from the tanks.

Daily levels of the following water parameters will be measured and recorded:

- o Salinity of tank water
- o Temperature of tank water
- o Dissolved oxygen content of tank water
- o pH of tank water.

The tank water will be aerated only when necessary to maintain the dissolved oxygen content above 60% saturation. The fish will not be fed for the duration of the test. A photoperiod of 16 hours light/8 hours dark will be maintained during the test.

2.6.5.C.8 Completion of Bioassay

After 96 hours, the tank water containing the sanddabs will be searched thoroughly for organisms. The fish will be considered alive if they show any response to prodding or gently swirling of the water. The number of live organisms will be counted and recorded.

2.6.5.C.9 Presentation of Data

If control mortality is greater than 10 percent, the results of the bioassay may not be evaluated. In this case, species selection and other test variables will be reevaluated in an attempt to reduce unacceptably high control mortality. If control mortality is less than 10 percent, the bioassay data will be presented in tabular form and will contain the following information:

- o Scientific name of test species
- o Number of fish in each treatment at test start
- o Number of fish alive at each observation period
- o Number of fish recovered alive at test end

- o Any behavioral abnormalities recorded.

2.6.5.C.10 Statistical Analysis and Interpretation of Results

If control mortality is less than 10 percent and is less than the mortality in the test material treatment, the test data will be statistically analyzed to determine if there is a significant difference in survival between control and test samples. The t-test (Snedecor and Cochran, 1980) will be used to compare the mean control and test survivals following the Levine's test for the homogeneity of sample variances.

If mortality in the test material exceeds 50 percent, an LC50 value (lethal concentration to 50 percent of the test organisms) will be calculated for any dilutions in which greater than 50 percent mortality occurs.

In the event that no mortality occurs in either control or test tanks, or that survival of organisms in the test tanks is equal to or greater than control organism survival, no statistical analyses will be performed.

3.0 TASK 2 - EVALUATION OF WHETHER PERSISTENT AND BIOACCUMULATIVE SUBSTANCE MAY BE ENTERING THE SAN FRANCISCO BAY FROM HPA

Section 3.5 Collection of Mussels from Uncontaminated Area

On page 3-3, Section 3.5, paragraph 1, sentence 3 is modified to read "The mussel shell length and mussel weight will be measured and recorded upon collection for size requirement verification and for later determination of growth following mussel deployment".

Section 3.7 Retrieval and Storage of Transplanted Mussels

On page 3-6, Section 3.7, a paragraph is added following paragraph 2 which reads "Mussel shell length and weight will be measured and recorded upon retrieval for comparison with mussel length and weight information obtained upon initial collection to determine mussel growth during the deployment period".

4.0 TASK 3 - EVALUATION OF STORM WATER RUNOFF TOXICITY

Section 4.4.1 Collection of Composite Storm Water Runoff Samples

On page 4-3 and 4-4, Section 4.4.1, paragraph 2, a sentence is inserted between the fourth and fifth sentence that reads "Storm water will be collected until cessation of runoff or until 8 hours elapsed time, whichever occurs first."