



ATTACHMENT

NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION  
AND  
CALIFORNIA STATE WATER RESOURCES CONTROL BOARD

MEASURES OF BIOEFFECTS ASSOCIATED WITH TOXICANTS  
IN SOUTHERN CALIFORNIA

YEAR TWO

PROPOSAL TO CONTINUE A COOPERATIVE AGREEMENT

JUNE 16, 1992

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**A. INTRODUCTION.** The State Water Resources Control Board (State Water Board) and the National Oceanic and Atmospheric Administration (NOAA) propose to continue a Cooperative Agreement to assess adverse biological effects in the inshore waters of Southern California. The program began in FY 1991-92 and this proposal is for FY 1992-93 (year two) with an option for a third additional year. In FY 1992-93, NOAA will provide \$250,000 to the State Water Board. The State Water Board will provide \$250,000 for the program from the Bay Protection and Toxic Cleanup fund. These funds will be used to determine the sediment toxicity of sites; determine the utility of using selected biomarkers; assess condition of selected fish species in the study area; and measure sediment chemistry on a limited number of samples.

This workplan is divided into several sections including Background, Rationale, Research Tasks, Project Deliverables, Benefits of the Research to NOAA, Benefits of the Research to California, Proposed Cooperative Agreement, Costs, and References.

**B. BACKGROUND.** NOAA is mandated by several acts of Congress to conduct a program of research and monitoring on marine pollution. Much of this research is being conducted through the National Status and Trends (NS&T) Program. The program performs regional intensive studies of the magnitude and extent of toxicant-associated bioeffects in selected coastal embayments and estuaries. The areas chosen for these regional studies are those in which the contaminant concentrations indicate the greatest potential for biological effects. These biological studies augment the regular chemical monitoring activities of the Program and provide answers to the proverbial "So what?" question regarding toxicants. NOAA and the State of California have begun to conduct one of these intensive programs in Southern California over a three-year period. This workplan describes the cooperative research effort to be conducted in the second year.

The State Water Board and its nine California Regional Water Quality Control Boards (Regional Water Boards) are mandated by the Porter-Cologne Act (California Water Code, Division 7, Sections 13390 et seq.) to implement the Bay Protection and Toxic Cleanup Program (BPTCP). One activity of the BPTCP is to develop sediment quality objectives. The intent of the sediment quality objectives is to protect the beneficial uses of bays and estuaries, including protection of human health and aquatic life. The objectives are to be based upon scientific information, including but not limited to chemical monitoring, bioassays, or established modeling procedures, and are intended to provide adequate protection for the most sensitive aquatic organisms. A strategy was approved for developing these objectives in July 1991. The strategy includes

upon selected coastal bays and lagoons. In the first year, samples were collected in Los Angeles/Long Beach Harbor, San Pedro Bay, Anaheim Bay, Alamitos Bay, and Huntington Harbour.

In the second year, samples will be collected in Tijuana Slough, San Diego Bay, San Diego Harbor, and Mission Bay. In the third year (pending availability of funds), it is anticipated that samples will be collected in Newport Bay, Bolsa Chica, Oceanside Harbor, and the numerous coastal lagoons located in the study area.

The research will involve biological and chemical analyses of sediments and resident demersal fish. Biological tests and chemical analyses will be performed with portions of each sample medium resulting in matching, paired data.

D. RESEARCH TASKS FOR FY 1992-93. Three research tasks are to be implemented in FY 1992-93: (1) measures of sediment contamination and toxicity; (2) measures of bioaccumulation and bioeffects in mussels; and (3) measures of bioaccumulation and bioeffects in resident demersal fish. The details of the technical approaches and methods to be used in these three tasks will be described by the State of California. Specific details of sampling schedules will be determined jointly by NOAA and the State of California.

Samples will be collected at sites in each embayment that will serve at least two purposes: (1) to characterize the magnitude and spatial extent of toxicant-associated bioeffects in Southern California inshore areas; and (2) to determine relationships between concentrations and mixtures of sediment-associated toxicants, bioavailability and uptake of these chemicals, and the occurrence and severity of bioeffects. It follows that the grid of sampling sites selected to fulfill these two purposes must meet the following criteria: (1) the sites must be depositional (muddy) and, therefore, should represent recently deposited toxicants; (2) half of the sites must represent the integrated accumulation of toxicants from multiple nearby sources, while the other half must represent contamination in further need of assessment for toxic hot spot status; (3) the grid of sediment sampling sites must be representative of conditions throughout the study area; (4) the grid of sampling sites must be suitable for estimating the spatial extent of toxicant-associated bioeffects; and (5) a subset of the sediment sampling sites must have marsh or demersal fish available. Based upon these criteria and the programmatic goals of NOAA's NS&T Program and the State Board's Bay Protection and Toxic Cleanup Program, a balanced sampling grid of sites in potentially highly toxic areas, in potentially moderately toxic areas, and in potentially reference (non-toxic) areas will be selected. Final selections will be performed jointly by NOAA and the State of California.

The data from the three years of research will be merged to form a synopsis of conditions in the study area. These data, in turn, will be compared with those from other parts of the study area that have been previously studied with similar methods. Data evaluations will be conducted jointly by NOAA and the State of California.

the collection of new data from California to verify toxicity thresholds previously determined in research performed in California and elsewhere. Matching, paired chemical and biological data will be collected in studies performed in California for analysis and evaluation. The BPTCP is also required to conduct monitoring for the purposes of (1) following up existing monitoring to determine whether these sites qualify as toxic hot spots and (2) sampling in previously untested areas to determine whether additional toxic hot spots exist. The program is beginning to implement this aspect of the BPTCP through a contract with the California Department of Fish and Game (CDFG).

C. RATIONALE. The proposed research will be performed in Southern California coastal bays. A considerable amount of research has been performed on toxicants and measures of effects associated with them in Southern California. Most of this work has focused upon Santa Monica Bay and the continental shelf off the large municipal treatment plants in the Los Angeles, Orange County, and San Diego metropolitan areas. A considerable amount of sediment chemistry data exist for the Los Angeles/Long Beach Harbor, parts of San Pedro Bay, and parts of San Diego Bay, the major embayments of Southern California. These data have been collected mostly as prerequisites to dredging projects. Sediment toxicity has been determined to a lesser extent in these embayments in a number of small predredging studies, but not in any large synoptic surveys. In Los Angeles/Long Beach Harbor, most of the sediment toxicity data are available for specific maritime berths and navigation channels. In San Diego Bay, much of the available data were generated in predredging studies or in studies performed with relatively insensitive species. No data are available from any synoptic survey of the harbor on a larger scale.

A program of cooperative research by NOAA and the State of California was initiated in March 1992 with FY 91 NOAA funds. It focuses upon the Los Angeles/Long Beach portion of the Southern California study area. That research included tests of sediment toxicity and toxicant-associated biomarkers in resident fish. This proposal focuses upon similar research in the San Diego area and is an extension of the first year of the overall research program. Toxicant-associated bioeffects are expected in the San Diego area since the concentrations of many chemicals have been reported as relatively high in some areas.

Objectives. The objectives of the research are:

- (1) determine the presence or absence of adverse biological effects in selected inshore and coastal areas of Southern California:
  - (a) sites which represent integrated conditions from multiple nearby sources of toxicants, and
  - (b) sites which qualify for assessment to determine hot spot status;
- (2) determine the relative degree of severity of toxicant effects;
- (3) determine the spatial distribution of toxicant-associated effects in selected areas of Southern California;
- (4) determine the relationships between toxicants and measures of effects in Southern California; and
- (5) determine the relative performance of a battery of biomarkers.

Scope of Study. The study area extends from the Palos Verdes Peninsula south to the Mexico/USA border. It extends from approximately the 60 m isobath to the upper limit of tidal-influenced saltwater; however, most of the work will focus

Sediment samples for possible future benthic community analyses will be collected at the same time the samples are collected for the toxicity tests and chemical analyses. At each station, 5 mini-cores (7 cm diameter, 5 cm deep) will be collected from a single deployment of the sampler. The entire contents of each core will be preserved and stored in formalin until taxonomic identifications are performed. NOAA and the State of California will determine jointly which samples, if any, will be examined for benthic community composition. Taxonomic identifications will not be a part of the present scope of work.

Toxicity Tests. Two independent tests of sediment toxicity will be performed with each sample: 10-day solid phase tests of the survival of amphipods (*Rhepoxynius abronius* or *Eohaustorius estuarius* (if reduced salinity renders *R. abronius* unusable); and pore water tests of sea urchin egg fertilization (e.g., *Strongylocentrotus purpuratus*, *Dendraster* sp., or *Lytechinus pictus*). The sediment from each station will be tested in the laboratory with five replicates. Each test will be accompanied with equivalent tests of a positive control chemical (e.g., cadmium chloride).

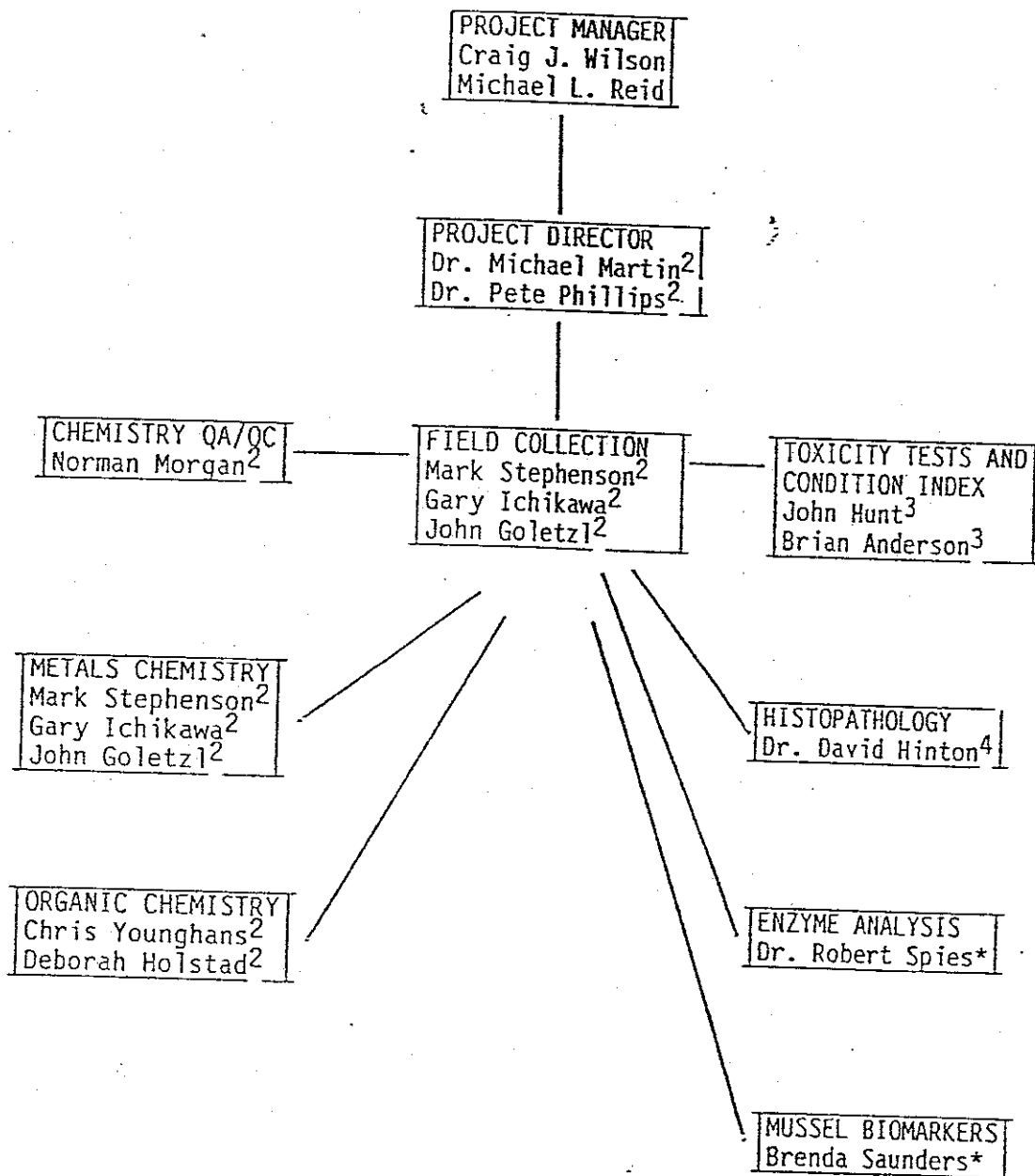
The amphipod tests will follow the protocols prepared by ASTM (1991) for performance of sediment toxicity tests with marine and estuarine amphipods. The end-point of survival will be reported. The urchin egg fertilization tests will be performed with the pore water (or interstitial water), which will be recovered using the protocols reported by Carr et al. (1989), and dilution series used by the NS&T study in Tampa Bay, FL. End-points of the urchin tests will include fertilization success (Dinnel et al., 1987). During the pore water tests, the concentrations of dissolved oxygen, ammonium, and hydrogen sulfide (if possible) will be determined along with pH, temperature, and salinity. The concentration on unionized ammonia will be calculated, based upon the pH and ammonium data, and reported.

Chemical Analyses. Portions of each of the sediment samples will be set aside and frozen for chemical analyses. Chemical analyses will be performed with as many of the samples as possible and feasible. Those samples that prove to be of interest to NOAA and the State of California following a review of the toxicity data will be analyzed for contaminant concentrations. The sediments from a minimum of samples (40) will be analyzed. Chemical analyses will be performed for the trace metals, pesticides, hydrocarbons, and selected normalizers (e.g., grain size, total organic carbon) that are routinely quantified by the NS&T Program, plus TBT and acid volatile sulfide. Analytical procedures will comply with NS&T Program QA/QC requirements and will include those for analyses of blanks and standard reference materials. In addition to the physical and chemical measures quantified by the NS&T Program, the concentrations of acid volatile sulfides (AVS) will be quantified as an aid in interpretation of the trace metals data.

Data Evaluations. The data will be evaluated to determine:

- (1) which stations and sites were statistically significantly more toxic than controls;
- (2) spatial patterns in toxicity;
- (3) relative degree of toxicity among the sites;

PROPOSED PROJECT ORGANIZATION CHART



1 State Water Resources Control Board  
 2 California Department of Fish and Game

performance of selected biomarkers in *M. edulis/galloprovincialis* or *M. californianus* in Southern California where the mixtures of toxicants may differ from those in the other two areas. Mussels are valuable biomonitoring organisms since they are sessile. Therefore, the data from analyses of mussels can be attributed to the specific sampling sites.

The cooperative agreement for the first year of research included a task to examine and evaluate candidate biomarker tests that may be applicable in the second year of the program. Based upon reviews of published articles and interviews with knowledgeable scientists, a battery of tests will be selected by the State Water Board and NOAA, in the first year and recommended for implementation.

**Sample Collections.** In this task, either *M. californianus* from Bodega Head (or some other suitable reference area) will be transplanted to a selected subset of the sediment sampling sites or resident bay mussels (*M. edulis/galloprovincialis*) will be collected at these sites. In these analyses of biological effects, emphasis will be placed upon resident mussels since they are most likely to represent long-term conditions at the sampling sites. At those sites in which resident mussels are not available for collection, mussels will be transplanted. The most feasible mussel transplants and collects will be timed to coincide with the annual sampling activities of the CMW Program (tentatively, September and December/January, respectively). Portions of the samples will be analyzed for toxicant concentrations and portions will be tested for a battery of biomarkers.

Sufficient numbers of mussels will be sampled at each site to allow determination of statistically significant differences among sites, based upon the site means. The tissues of a sufficient number of animals will be composited from each site to provide the minimum amount needed for the full suite of chemical analyses.

Mussels will be collected, handled, and transplanted using standard, non-contaminating methods of the CMW Program. All biomarker and chemical analyses performed on transplanted mussels also will be conducted on control mussels collected at Bodega Head.

The number and final locations of sampling sites will be determined jointly by NOAA and the State of California. Mussels will be transplanted to and retrieved from these sites in the same sequence. Portions of the samples will be allocated for the biomarker and chemical analyses and transported to the participating laboratories as quickly as possible.

**Biological Tests.** The biomarkers being considered in the first year of the program as candidates for implementation in the second year were to include:

- (1) micronuclei formation (Brunetti et al., 1988) in gill epithelium;
- (2) DNA unwinding (Nacci and Jackim, 1989; Shugart, 1988) in gill epithelium;
- (3) histopathological disorders (including neoplasia) in gills, gonads, hemocytes and digestive glands (Gardner and Yevich, 1988);
- (4) condition index (Crosby and Gale, 1990); and
- (5) gonadal index (Galtsoff, 1964) and spawning condition;



I. COST ESTIMATES FOR FY 1992-93 NOAA COOPERATIVE AGREEMENT.

Task 1. Survey of Sediment Contamination and Toxicity

Sample Collection

40 study sites @ \$1425  
15 reference sites @ \$1425

\$ 57,000  
21,375

Toxicity Testing (2 tests)

120 study stations @ \$1000  
45 reference stations @ \$1000

120,000  
45,000

Chemical Analysis

40 study stations

full organic scan + PAH @ \$1180  
full metal scan @ \$900  
TBT @ \$170  
TOC @ \$50  
Grain size @ \$40  
AVS @ \$340

47,200  
36,000  
6,800  
2,000  
1,600  
13,600

Reports

5 Cruise @ \$500  
4 Quarterly @ \$500  
1 Data report @ \$1250

2,500  
2,000  
1,250

Miscellaneous Services, expendables (pore water extractions  
for the urchin test, benthic archive, additional chemical  
analyses for NS&T, etc.)

23,675

\$380,000

Task 2. Bioaccumulation and Biomarkers Study  
(Sample collections, biomarker tests,  
chemical analyses, data evaluations, etc.--  
please refer to text for explanation.)

\$120,000

\$500,000

The procedures to be used in the data evaluations will be determined jointly by NOAA and the State of California. The relative sensitivity, range in response, within-site variability, and concordance with tissue chemistry data will be determined for each biomarker. Sampling sites at which mean results are significantly different than controls will be identified. Mean results will be used to determine spatial patterns in response among the sampling sites.

#### E. PROJECT DELIVERABLES.

Products to be delivered to NOAA include:

- o Cruise report which will consist of station locations with longitude and latitude readings for each station and site, a chart indicating the location of all stations and sites, and field notes regarding the sampling success and visual condition of the samples.
- o Quarterly progress reports.
- o A draft and final technical report of second year work which will include description of methods, raw data in tabular form, the results of the data evaluations, and textual descriptions of the results.

#### F. BENEFITS OF THE RESEARCH TO NOAA.

This research program in Southern California will provide a number of programmatic benefits to NOAA. They include:

- presence or absence of adverse biological effects in areas known to have relatively high chemical concentrations;
- data to assess the degree or severity of toxicant effects;
- spatial distribution of toxicant-associated effects in Southern California;
- new data with which to supplement existing data on the relationships between toxicants and toxic effects in Southern California;
- matching biological and chemical data with which to perform statistical analyses;
- biological data with which to assess the significance of chemical data from the NS&T Program's monitoring activities; and
- evaluations of the relative performance of a battery of biomarkers.

#### G. BENEFITS OF THE RESEARCH TO CALIFORNIA.

The benefits of this program to the State of California would be similar to those to NOAA:

- presence or absence of adverse biological effects in areas known to have relative high chemical concentrations;
- data to assess the degree or severity of toxicant effects;
- spatial distribution of toxicant effects in Southern California;
- new data with which to supplement existing data on the relationships between toxicants and toxic effects in Southern California;

in erythrocytes and reduced reproductive success have been reported in white croaker collected off the Palos Verdes Peninsula (Hose et al., 1987). Marsh fish generally are territorial in behavior and have a small range; therefore, data from analyses of these fish can be attributed to the sites in which they were sampled. White croaker are more mobile and have a wider range.

In the first year of the program, demersal gobies will be collected at selected sites and analyzed for selected biomarkers and chemical concentrations. Candidate species in the first year included Lepidogobius lepidus, Ilypnus gilberti, and Clevelandia ios. These species burrow into sediments and are territorial. Often, they are abundant. A methods evaluation step is scheduled to be conducted in the Los Angeles area in the first year, candidate biomarker tests will be performed and the results evaluated. Based upon the results of the evaluation step, a survey of several sites will be conducted using the selected biomarker tests. The results of the first year's work will be evaluated jointly by NOAA and the State of California to prepare a similar survey plan for the San Diego Bay area in the second year.

In addition, the incidence of histopathological disorders in the livers of fish collected in the first year (which was deferred) will be determined as a part of the second year of research.

Sample Collections. Fish will be collected at selected sites plus a pristine control site to be specified. They will be caught with trawls as specified in the first year. At least the minimum number of fish necessary to perform the biological and chemical analyses listed below will be captured at each site. Portions of the fish will be allocated for each of the analyses. The gall bladders of the fish will be shipped to NOAA/NMFS in Seattle for possible analyses of PAH metabolites in the bile.

Biological Tests. As a minimum, the biological tests under review in the first year include:

- (1) condition index;
- (2) gonadal/somatic index;
- (3) cytochrome P-450 and EROD induction in livers; and
- (4) neoplasms and other histopathological disorders in the livers and kidneys.

Chemical Analyses. The liver tissues of the fish will be analyzed for the trace metals, pesticides, hydrocarbons, and normalizers routinely quantified in the NS&T Program.

Data Evaluations. The data will be evaluated to determine:

- (1) relative bioaccumulation of sediment-associated toxicants in the tissues of the fish;
- (2) the relative performance of each of the biomarkers;
- (3) the presence/absence of statistically significant results among sites and between sites and reference or control sites;
- (4) the relative degree or severity of effects observed in each site; and
- (5) the relationships between the chemistry and biological data.

- (6) heat stress protein induction (Sanders, 1990);
- (7) glutathione content and/or glutathione-s-transferase activity (Lee et al., 1988).

Chemical Analyses. Tissues from mussels collected at each site and from Bodega Head will be analyzed for the trace metals, pesticides, hydrocarbons, and normalizers quantified by the NS&T Program. Standard methods that comply with the NS&T Program QA/QC requirements, including analyses of blanks and standard reference materials, will be used during the analyses.

Data Evaluations. The data will be evaluated to determine:

- (1) the concentrations of toxicants in transplanted or resident mussels;
- (2) the relative performance of each of the selected biomarkers;
- (3) the quantification of mean biomarker results at each site and the identification of significant differences among sites and between the study sites and the controls;
- (4) the geographic patterns in the incidence of the measured biological effects; and
- (5) the relationships between the biological and chemical data.

The statistical methods to be used in the data evaluations will be determined jointly by NOAA and the State of California. The chemical signatures and absolute concentrations of contaminants in the sediments at each site will be compared with those in the mussels collected at the same sites. The relative sensitivity, range in response, within-site variability, and concordance with the tissue chemistry will be evaluated for each of the biomarkers. Analyses will be performed to identify at which sites, if any, the mean results were significantly different from those in Bodega Head controls. The mean results will be evaluated to identify spatial patterns in results among the sites. The chemical data from the tissue analyses and the sediment analyses (from Task 1) will be compared with the biomarkers data to identify any relationships and to conform concentrations predicted by sediment-water and water-tissue equilibrium partitioning models.

Deliverables. Products to be delivered to NOAA by the State of California will include detailed descriptions of methods, the raw data in tabular spreadsheet format, the results of the data evaluations listed above, and textual descriptions of the results.

#### b. Bioaccumulation and Biomarkers in Marsh and/or Demersal Fish.

Rationale. The tests of sediment toxicity will provide some information on the relative bioavailability and toxicity of sediment-associated toxicants to benthic animals. The analyses of mussels will provide information on uptake and bioeffects in immobile, transplanted mollusks whose recent history has been documented. But neither task will provide information on uptake and effects in resident, demersal, feral animals closely associated with sediments. Marsh fish (*Fundulus heteroclitus*) have been used elsewhere by other investigators (e.g., Binder and Stegeman, 1980) to determine adverse responses of resident fish to exposure to toxicants. Histopathological disorders have been observed in white croaker (*Genyonemus lineatus*) collected in San Pedro Bay and other locations in Southern California (Malins et al., 1987). Elevated incidences of micronuclei

- matching biological and chemical data with which to perform statistical analyses;
- biological data with which to assess the significance of chemical data from the Mussel Watch monitoring activities;
- evaluations of the relative performance of a battery of biomarkers; data to be used in a research program to develop State sediment quality objectives; and
- data to be used in a regulatory program to identify toxic hot spots.

H. PROPOSED COOPERATIVE AGREEMENT. This research will be implemented through a Cooperative Agreement between NOAA and the State of California. NOAA will transfer \$250,000 to the State Water Board, and the State Water Board will provide \$250,000 for a total budget of \$500,000.

Responsibilities for overall programmatic and technical direction will be shared by both NOAA and the State Water Board. The State Water Board will disperse the merged funds to their prime contractor, CDFG, for task implementation. If needed, either the State Water Board or CDFG will acquire certain skilled services from investigators at other State universities, agencies, and laboratories for implementation of the research. CDFG will be the principal subcontractor to NOAA for the project. Research results and products will be developed for review and access by all three agencies. All three agencies will provide technical staff support for program planning, determination of technical scope and methods, logistics planning and facilitation, data evaluation, report review, and agency interface.

Collaboration with other NOAA components and other federal agencies will be handled by NOAA staff, while State of California interagency arrangements will be handled by the State Water Board.

- (4) relationships between the toxicity and chemical data; and
- (5) relative sensitivity of the two toxicity tests.

Statistical methods to be used for identification of significantly toxic sites will be chosen jointly by NOAA and the State of California. The relative degree of toxicity will be determined according to the mean results for each station and site and reported graphically and in tabular formats. The relationships between the toxicity and chemical data will be determined in regression analyses, cluster analyses, concordance tests, and other methods to be specified. In addition, the bioassay and chemical data will be entered into a project data base and accumulated in that data base. As each leg of the study plan is completed, the new data will be added to this data base. Similar data collected elsewhere in the study area simultaneously with this task also will be entered into the data base. Other similar data from dredging programs and other research programs previously conducted in the study area by others also will be acquired and entered into this file. These matching data will be available for further evaluation to determine patterns in concordance, co-occurrence in sediment contamination and toxicity, apparent effects threshold, no-effects levels, etc.

Deliverables. Products to be delivered to NOAA by the State of California will include the descriptions of methods, the raw data in tabular spreadsheet format, the results of the four data evaluations listed above, and textual descriptions of the results.

## Task 2: Bioaccumulation and Biomarkers Study

Based upon the results of the year-one analyses and reviews of biomarkers and bioaccumulation, the State Water Board and NOAA will jointly select the specific focus of the year-two work. Depending on year-one results, a determination will be made to proceed with Tasks 2.a. (mussels), 2.b. (marsh and/or demersal fish), or a combination of these tasks.

The biomarker tests to be performed will be selected jointly by NOAA and the State of California. In the second year, some of the selected tests will be conducted at selected sites within the study area.

### a. Bioaccumulation and Biomarkers in Mussels.

Rationale. Mussels (*Mytilus californianus*) are collected from Bodega Head, transplanted to selected coastal sites, retrieved several months later, and analyzed for the occurrence of selected toxicants by the State of California Mussel Watch (CMW) Program. Also, NOAA performs similar analyses annually of the tissues of resident mussels (*M. edulis/galloprovincialis* in bays or *M. californianus* along the coast) collected from many locations in California as a part of the NS&T Program. The concentrations of some chemicals in the tissues are sufficiently high in some sites to warrant concern that adverse effects are occurring but very little effort has been expended to determine if these animals are suffering any adverse effects.

Along with the evaluations of reproductive success and biomarkers in mussels (*M. edulis*) in Buzzards Bay/Boston Harbor (J. Cappuzzo, WHOI) and in oysters in Tampa Bay (W. Fisher, EPA) supported by the NS&T Program, we will evaluate the



## Task 1. Survey of Sediment Contamination and Toxicity

Rationale. Sediment toxicity tests provide a direct means of determining the relative biological significance of sediment-associated contaminants. Sediment-associated toxicants can be assumed to be bioavailable and concentrated to unacceptable levels when toxicity tests demonstrate that the sediments are toxic. Standardized methods have been developed and are available for use in these tests.

Sample Collections. In this task, surficial sediments (upper 2 cm) representative of selected bays will be collected for chemical analyses and toxicity testing. The number of sampling sites in each embayment will be tailored to the size and configuration of that bay. For comparative purposes, selected sampling sites along the coast seaward of the selected bays and comparable to the study sites in grain size and organic content also will be sampled. Three stations per site will be sampled and tested independently. The three stations at each site will be arranged in either triangular or linear configurations with the stations located up to 200 m apart. Only fine-grained, depositional sediments will be collected. Half of the sampling sites will represent integrated conditions from multiple nearby sources of toxicants; the other half will represent contamination in further need of assessment for toxic hot spot status, regardless of the variety and source of toxicants. A Kynar-lined, modified Van Veen grab, or box core will be used to collect sediments using methods that will not contaminate the samples.

Multiple deployments of the sampler will be required to obtain sufficient material at each station for the battery of toxicity tests and chemical analyses. Care will be taken to ensure that cross-contamination between samples does not occur. After completing sampling at each site, the sampling devices and utensils will be thoroughly washed with seawater and hexane between sites and washed with seawater between stations.

A total of 120 samples (40 sites X 3 stations) will be sampled within the second year study area, plus the reference and control sediments. NOAA and the State of California will jointly select the number and final locations of the sampling sites. The exact coordinates for each site will be determined at that time using the five criteria described above.

The sampling sites will be sampled in multiple legs, each leg consisting of 5 to 10 sites. The reference sediments and controls will be sampled and tested with each leg. Reference sediments will be collected from locations in California that are relatively unpolluted, not toxic, and comparable to the study sites in grain size and organic content. Control sediments will be collected from locations such as the home sediments of the amphipod test animals that are also unpolluted and not toxic.

The chain of custody will be documented during sample handling and shipment. Sediments will be kept cool (4°C) during shipping and not held for more than 10 days before toxicity tests are initiated. All procedures will comply with the NS&T Program QA/QC requirements.



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