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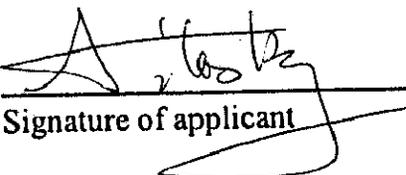
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Silas S.O. Hung

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II. Title Page

Chronic Toxicity of Environmental Contaminants in Sacramento Splittail (*Pogonichthys macrolepidotus*): A Biomarker Approach

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Type of Organization and Tax Status:

University of California, Davis

1. Executive Summary

Chronic Toxicity of Environmental Contaminants in Sacramento Splittail (*Pogonichthys macrolepidotus*): A Biomarker Approach

Silas Hung Ph.D., Swee J.Teh Ph.D., and Jay A. Davis PhD

The maintenance of a population of fishes is heavily influenced by the constantly changing physical and biological conditions of the ecosystem. Contaminant stress may compromise the ability of fish to survive natural environmental stressors. Ultimately, fish populations that are unable to compensate for additional stress will show reductions in growth, reproductive capacity, and survival. For a species that has already declined drastically, survival and reproductive success have profound ecological significance. When exposed to various stressors such as from contaminants, the fish may not even survive to carry out the ultimate function of reproduction. Furthermore, chronic dysfunctions of reproduction in the surviving fish might ultimately result in decreased fecundity and fertility, and thus indirectly impacting the population level. Although water quality deterioration, water diversions, and habitat loss and degradation (all resulting from human activity) may have contributed to declines in fish population, there is a high degree of certainty that splittail population is adversely affected by exposure to contaminants from commercial, domestic, and agricultural sources. However, integrated laboratory and field investigations using biomarker approach to detect and quantify chronic contaminant responses in splittail are lacking.

The Sacramento splittail (*Pogonichthys macrolepidotus*), a Federally listed threatened species, forages on benthic organisms. Splittail are now largely confined to the Sacramento-San Joaquin Delta, Suisun Bay, Suisun Marsh, the Napa River, and the Petaluma River except during spawning migrations in winter and spring (Meng and Moyle, 1995). Juvenile remain in the river system for weeks to a year before migrating to the delta where they mature at age 2. Adult fish remain in the bay-delta system year round but will migrate upstream to spawn in fresh water preferably over flooded vegetation. Unlike other native fish species where laboratory culture is difficult and expensive, splittail is easily cultured at low cost and will spawn in captivity providing access to all life stages. Therefore, we propose to use splittail as a native fish model for chronic toxicity study.

We will demonstrate the use of biomarkers (biological responses), in conjunction with ongoing biomonitoring efforts of fish population by DFG and water, sediment, and tissue contaminant monitoring by SFEI and USGS, to evaluate the chronic effects of contaminants on the health of splittail under laboratory and field conditions. Four functional categories of biological indicators will be measured: 1) indicators of contaminant exposure, 2) indicators of general condition indices, 3) indicators of organ and reproductive dysfunction, and 4) indicators of individual-level response. We propose to evaluate a suite of biomarkers of exposure and effect indicators at several levels of biological organization to quantitatively: 1) assess the potential chronic effects of contaminant exposure on various life stages of splittail under laboratory and field settings, 2) establish a link between the contaminant exposure and the deleterious health (growth and reproduction) of individual splittail, and 3) identify indicators of contaminant exposure that are most cost-effective for use in future monitoring studies. This will provide valuable information for future environmental compliance and regulatory studies and the ecological risk assessment process. These studies also have practical application as laboratory data linking specific contaminants with adverse chronic effects (as well as field data correlating biomarker expression with contaminant exposure) that could help guide management decisions with respect to determining acceptable contaminant levels in the environment.

Three tasks will be conducted simultaneously for three years. In task 1, we will investigate

adult and juvenile splittail from several field sites and compare a range of biomarkers of exposure and adverse effects in individual splittail as a function of the site. Concentrations of mercury (Hg), selenium (Se), diazinon, polychlorinated biphenyl (PCBs), and organochlorine pesticides will be measured in gonads and whole bodies of 5 fish from each site. This chemical monitoring will be closely integrated with other SFEI projects (RMP, SRWP, CISNET, and fish sampling for the CALFED designated action on mercury) that will provide additional information on concentrations of these contaminants in water, sediment, and fish tissue at some of the study locations. Embryos will be collected and grown in a clean environment for developmental toxicity study. We will select sites along the Sacramento River, the Sacramento River in the vicinity of the Yolo and Sutter bypasses, and Suisun Bay/marsh where contaminants such as Se, Hg, diazinon, pyrethroid, and PCBs are known to be present.

In task 2, we will investigate a range of biomarkers of exposure and adverse effects in laboratory-raised splittail. We plan to expose all life stages (embryo/larvae, juvenile, and adult) to one or several reference toxicant(s) at concentrations within the range determined in field samples. Laboratory exposures will be 7 days for embryo and 30 days for juvenile and adult. Metals (selenium and mercury) and pesticides (diazinon and pyrethroid) will be used. After exposure, all fish will be transferred to clean water. Embryo and juvenile groups will be sacrificed at Day 0, 7, 14, 21, and 28 post exposures. Adult groups will be sacrificed at day 0, 90 and 180 post exposures.

In task 3, we will review existing contaminant-related literature pertaining to our experiments on splittails. Correlation of the integrated biomarker of responses and the spatial patterns of contaminant concentrations in tissue, water, and sediments will be determined using multivariate statistical analysis. Write and submit quarter and annual reports of findings to CALFED. Manuscripts from the proposed research will be submitted to a high quality scientific journals for peer review and publication. Results will be disseminated widely through participation in workshops and seminars, and presentation of papers at an international/national meeting.

Dr. Silas Hung has 20 years experiences in fish rearing and nutrition under laboratory condition. Dr. Swee J. Teh has 12 years of extensive field and laboratory research experience on ecotoxicology and biomarker study. He has also experienced in projects and experimental design and managing contracts and grants' total >\$1 millions per year. Dr. Jay Davis assists with management of the Regional Monitoring Program for the San Francisco Estuary and is the principal investigator in several studies of fish contamination in the Sacramento River, the Delta, and the Bay. Our experience in field sampling and evaluation, biomarker approach, aquatic toxicology, biochemistry, fish nutrition, fish pathology, and husbandry will facilitate completion of this study.

Local support/coordination: This project support ongoing efforts by the USFW Service and the IEP in recovering threatened and endangered fish populations in the Sacramento-San Joaquin system by facilitating restoration planning and monitoring. We will be working with DFG's splittail monitoring survey, IEP funded splittail culturing projects, and various water quality monitoring programs (e.g. DFG, SWRCB, SFEI, USGS).

Budget Costs and Third Party Impacts: To ensure the completion of this combined field and laboratory chronic toxicity study, we are requesting a total funding of \$ 673,684.00 for three years. Task 1 and 2 can be separated if funding is limited.

There are no third party impacts.

2. Project Description

2.1. Proposed Scope of work

There is a high degree of certainty that resident fish populations in California are adversely affected by exposure to contaminants in the environment. However, investigations to detect and quantify chronic sublethal responses in fish of California watersheds that are attributable to contaminants are lacking. Risks to fish populations statewide are difficult to quantify because integrated laboratory and field data are lacking. The objective of this study is to utilize ongoing efforts by DFG in assessing the splittail population, along with USGS and SFEI monitoring data of contaminant levels in water, sediment, and tissue samples, and combines it with University of California-Davis (UCD) expertise in fish nutrition, biochemistry, husbandry and Toxicopathologic Consulting expertise in biochemical and histopathological biomarkers to help establish a causative link between contaminant exposure and chronic deleterious health effect in splittail. We propose a 3-year extensive field and laboratory investigation of all life stages (embryos/larvae, juveniles, adults) of splittail where four functional categories of biological indicators will be measured (Table 1). The integrated biomarker approach is based on using key aquatic organisms and specific responses of these organisms as integrators. Both short-term indicators such as biochemical and histopathological responses and longer-term more ecologically relevant indicators such as growth and reproductive responses are included in the biomarker approach. Thus, this approach allows for the short-term sensitive biological responses (biomarkers) to be linked or correlated to significant longer-term individual effects (growth and reproduction). In this manner, cause and effect relationships between environmental stressors (e.g. contaminants) and ecologically relevant responses (e.g. population declines) can be established. This will provide valuable information for future environmental compliance and regulatory studies and the ecological risk assessment process.

Table 1. Four functional categories of biological indicators and the biomarkers of exposure and adverse effects in field and laboratory investigation.	
Biological indicators	Biomarkers methods and significance
Indicator 1. Indicators of contaminant exposure	body burdens of contaminants, biochemistry (detoxification enzymes) and histopathology (toxicity-related lesions)
Indicator 2. Indicators of fish condition indices	glycogen & lipid contents, length and weight, liver and gonadal somatic indices, and gross morphological abnormalities
Indicator 3. Indicators of organs and reproductive dysfunctions	blood chemistry (vitellogenin for endocrine disruption), immunohistochemistry (P450, Stress proteins, metallothionein), histopathology (intersex and tumors)
Indicator 4. Indicators of individual-level responses	age, growth (time to mature), fecundity and fertility (time to hatch, % hatch, viability, and survival)

Task 1: Assessment of wild splittail for body contaminant burdens and four functional categories of biological indicators. (3 years).

We propose to investigate splittail from three sites and compare a range of biomarkers of exposure and adverse effects in individual organisms (Table 1) as a function of the site. We will sample 30 juveniles and 30 adult using short gill net sets, beach seines or hook and line between June and September at locations near Suisun Marsh, Suisun Bay, Sherman Lake, Nurse Slough, and Sacramento River below Rio Vista and Big Break. Gonads of the adult begin to mature around September-October and fish will be spawn out around March -April.

1) Adult/embryos: 20 adults will be sacrificed immediately for biomarkers approach (Table 1), the other 10 will be transported to UCD for study of developmental toxicity. If for unknown reasons where only 10 or fewer adults were collected, our priority will be to transport all the fish to UCD instead of sacrifice the fish immediately. For biomarker study (Table 1), a dorsal fin ray will be collected for age determination. Observations will be recorded for general conditions, such as presence or absence of fin disease, body and/or mouth sores, internal and external parasites. Fish will be weighed and measured for a condition index ($CI = 10^5 W/L^3$, W=body weight and L = total length), blood will be collected from adults for biochemical analysis, liver and gonads will be weighed to determine liver and gonadal somatic indices and fixed in 10% buffered formalin for immunohistochemical, and histopathological analyses (Teh *et al.*, 1993; 1997; 1999). Sections of liver will be frozen for detoxification enzymes analysis. Concentrations of mercury, selenium, diazinon, PCBs, and organochlorine pesticides will be measured in gonads and whole bodies of 5 fish from each site. This work will be closely integrated with other SFEI projects (RMP, SRWP, CISNET, and fish sampling for the CalFed designated action on mercury) that will provide additional information on concentrations of these contaminants in water, sediment, and fish tissue at the Petaluma River, Napa River, Suisun Bay, Sacramento River, and possibly other locations. For the developmental toxicity study, fish from each site will be reared in separate tanks, embryos will be collected and examined under a dissecting microscope for developmental dysfunctions. Intermittently, 15 embryos from each site will be fixed in 10% buffered formalin for histopathological analysis of developmental defects not detected by gross examination. The % of hatches, time to hatch, viability, and survival rates of embryos from each site will also be assessed (Table 1). Spawn adult will be sacrificed and processed for biomarker study as described above.

2) Juvenile: 30 juveniles will be collected in the similar areas as the adult between June and September with beach seines. Fish will be processed immediately and a range of biomarkers of exposure and adverse effects discussed in task 1 will be assessed (Table 1).

The significance of this field study is to derive a pattern of biomarker indicators of responses that are related to contaminant exposure (Table 1). A multivariate statistical analysis integrating the four biological indicator responses as a function of each site will be compared to laboratory-exposed groups and to groups from other field sites.

Task 2: Assessment of laboratory splittail for body contaminant burdens and four functional categories of biological indicators (table 1).-3 years

Laboratory exposures will use representatives of major contaminant groups determined in field study (Task 1). To prevent overloading of work and to provide a more focused study, we will be targeting metals [selenium (Se) & mercury (Hg)], an organophosphate pesticide (diazinon), and pyrethroid pesticide (permethrin) (CalFed ERP, Vol II). Embryos, juveniles and adults will constitute the F2 generation of wild splittail originally captured from the Sacramento Delta. Fish will be weaned to the standard purified diet developed by Dr. Silas Hung. Daily monitoring will include recording of water temperature, salinity, and mortality. Water quality (O_2 , ammonia, nitrate, nitrite, pH) will be assessed on a weekly basis.

1) 900 exposed/180 control embryos: Three replicates of 60 embryos per replicate (5 chemicals and one control) will be bath exposed to Se, Hg, Se/Hg, diazinon, permethrin, and control water for 7 days (d) or until hatched. Observations will be recorded for general conditions, such as mortality, % hatched and survival. Embryos will be examined under a dissecting microscope for developmental abnormalities. At day 5 during the exposure, 10 embryos per group will be fixed in 10% buffered formalin for histopathological analysis of lesions that were not detected by gross examination. After

exposure, hatchlings will be transferred to clean water. At day 0, 7, 14, 21, and 28 post exposure, 10 larvae per replicate from each group will be sampled for developmental toxicity study. Fish will be weighed and measured for a condition index and a range of biomarkers of exposure and adverse effects will be assessed (Table 1).

2) Juvenile and Adult (450 exposed/90 controls): Juvenile and adult exposures will be dietary for metals (30 d) or aqueous for pesticides (7 d). Juvenile (6 month old) and adult (age 2-3) will be exposed to Se, Hg, Hg/Se, diazinon, and permethrin during the month of September/October (pre-spawning period and during gonadal development). At day 0, 90, and 180 (the day after fish spawning), 30 juveniles and 30 adults will be sampled (10/replicate for 3 replicates/exposure). A range of biomarkers of exposure and adverse effects similar to task 1 will be assessed (Table. 1). Embryos will be collected from the exposed adult, developmental toxicity study (described in Task 1) will be followed. Body burdens will be analyzed to determine levels of contaminants. A subset of juvenile exposed to Se, Hg, and Se/Hg mixtures will be reared until sexually mature for fecundity and fertility study. Embryos will be assessed as described in task 1.

The significance of this field study is to derive a pattern of biomarker indicators of responses that are related to the specific contaminant exposure. It is anticipated that a multivariate statistical analysis integrating the four biological indicator responses as a function of each laboratory exposure will be assessed and compared to those of field studies (Task 1).

Task 3: Integrating field and laboratory study. (3 years)

We will review existing contaminant-related literatures pertaining to chronic toxicity of splittail. Data will be entered into a spreadsheet program (Microsoft Excel) and sorted on the basis of gender, organ, and treatment group. Average scores for each lesion will be compared using Mann-Whitney Rank Sum Test between sites and experiments. Analysis of variance (ANOVA) will be used to test site and laboratory exposure effects. In the final year, correlation of the four functional categories of biological indicators, DFG splittail population monitoring data, and spatial patterns of contaminant concentrations in tissue, water, and sediments will be integrated using multivariate statistical analysis to determine relationship between the contaminant loading in the environment and fish health. The growth and reproductive success will be monitored to determine the chronic effects of contaminant exposures. Quarterly and annual reports of findings will be submitted to CALFED. Manuscripts from the proposed research will be submitted to a high quality scientific journals for peer review and publication. Results will be disseminated widely through participation in workshops and seminars, and presentation of papers at international and national meetings.

2.2. Location and/or Geographic Boundaries of the project.

1. Field study: The populations of splittail in the delta and north San Pablo bay are believed to represent separate populations (R. Baxter). We will select appropriate sampling sites along Sacramento-San Joaquin river, the Sacramento River in the vicinity of the Yolo and Sutter by-passes, Suisun Bay/Delta and San Pablo Bay where two separate populations of splittail will be targeted and contaminants such as selenium, mercury, diazinon, pyrethroid, and PCBs are known to present.

2. Laboratory study: The laboratory study will be carried out in the Aquaculture and Fisheries Program (AFP) and the Animal Science Nutrition Laboratory (ASNL) of UC Davis. Fish will be maintained at the AFP facility which is equipped with a 1500 ft² metal quonset hut and a 800 ft² indoor preparation laboratory for biological sample preparation. The ASNL laboratories are equipped with scales, refrigerators, freezers, tissue processing equipment and scientific instruments needed for biochemical and histopathological determination. Laboratory and university personnel monitor these facilities 24 hours daily.

3. Ecological/Biological Benefits

3.1. Ecological/Biological Objectives

Environmental managers and regulators are searching for better, more cost-effective ways to assess the impacts of contaminants on aquatic organisms, to restore the formerly contaminated watersheds, and to protect existing watersheds. Fish population monitoring is an important and necessary means of evaluating the effects of contaminants on aquatic ecosystems and of prioritizing future cleanup efforts. While acute toxic effects (e.g., fish kills) caused by releases of hazardous substances into waterways are easily perceived and quantified, chronic toxic effects are not as easily recognized and assessed. For example, pulsed or continuous low-level contaminant releases can generate multiple insidious adverse responses in resident fish populations that are not visible to the naked eye. These responses include degeneration of vital organs, tumors, suppressed immune function, disease, endocrine disruption, reproductive malfunctions, behavioral alterations, and developmental defects in young. Any one or a combination of the responses can ultimately lead to a reduction in the abundance and diversity of fish populations in a contaminated watershed.

Sublethal effects or "biomarker" monitoring offers a new and necessary approach for identifying and assessing the chronic toxic effects of contaminants on resident fish populations. Biomarkers are biochemical, physiological, or histopathological indicators of exposure to and/or effects of contaminants at the suborganismal or organismal level. General types of biomarkers include measurements of genetic, metabolic, immunologic, histopathologic, and physiologic processes (Huggett *et al.*, 1992). Biomarkers have been used extensively to evaluate the exposure and effects of multiple environmental contaminants (Teh *et al.*, 1997; Adams *et al.*, 1999). Biomarkers reflect the bioavailability of contaminants, provide a rapid and inexpensive means for toxicity assessment, and serve to fingerprint specific classes of chemicals responsible for adverse effects. Advantages of biomarker monitoring include (1) biotic integration of diverse exposure pathways and temporal variability; (2) ability to integrate responses across multiple chemical and environmental stressors; and (3) cost effectiveness relative to extensive chemical analyses (Anderson *et al.*, 1997). Finally, biomarkers can serve as an "early warning" system for detecting the adverse effects of contaminants on resident fish populations (Adams *et al.*, 1999).

3.1a. Selection of Indicator Species: Splittails were selected as a fish indicator species because of the low cost of culturing and fish will spawn under captivity hence providing easy access to all life stages. Recent studies sponsored by the San Francisco Estuary Project (1995) have shown that many native benthic invertebrates in the San Francisco Bay and the Delta are being replaced by non-native species that bioconcentrate metals and organic contaminants to significantly higher levels than native invertebrates. There is a concern of the potential exposures of splittail to mercury, selenium, and organochlorine compounds that are known to affect the reproductive ability of other fish species (Brooks *et al.* 1997; Mac *et al.* 1993;). Although on-going studies by the DFG are attempting to assess the abundance and fecundity of splittails, no study has yet correlated evidence of reproductive impairment to contaminants exposure.

3.1b. Selection of contaminants: Water quality in the Sacramento-San Joaquin ecosystem has been impacted by contaminants from multiple sources that include agricultural runoff, mining wastes, paper and pulp mills, oil spills and oil refinery discharges, hazardous material spills, numerous permitted point sources such as petroleum refineries, and storm water runoff (sections 4, 5, and 8 of Water Quality Program, Pp 40 of ERP V-I). As many as 65 pollutants, primarily pesticides, metals, PCBs, and PAHs, have been identified as entering the San Francisco Bay estuary at a rate of 5 to 40,000 tons per year (SFEI 1991). Contaminants known to be elevated above EPA water quality criteria

(SOME OF THESE CRITERIA ARE BASED ON HUMAN HEALTH) include chromium, copper, nickel, lead, selenium, mercury, PCBs, PAHs, DDTs, chlordanes, dieldrin and diazinon (SFEI 1995). Because of the complex ecosystem, it is difficult to study every single or a mixture of contaminants under laboratory settings. Therefore, we will select two metals (Se and Hg) and two pesticide (diazinon and permethrin) that have been extensively monitored and reviewed in California (Water Quality Program, Pp 40 of ERP V-I). The purpose is to derive a pattern of biomarkers that we can compare to field sample where the contaminants were predominantly found.

Our objective is to combine in-depth field surveys with controlled laboratory exposures, and an overall biomarker approach, to establish a causative link between contaminant exposure and deleterious effect in splittail. Working in concert with DFG (funded by IEP) to assess the splittail population, we will obtain field samples from sites along the Sacramento-San Joaquin estuary including San Francisco Bay delta, Yolo-bypass, and contributing streams. At the same time, an extensive laboratory exposures will be conducted. The laboratory study will evaluate the utility of biomarkers (used singly and in combination) in assessing contaminant impact on splittail, and will generate data which can form the basis for a model system defining transport, fate, and impact of contaminants. Laboratory exposures will refine assay protocols, assess biomarker sensitivity and specificity, and define target organs and biological effect. Field surveys will allow biomarkers to be correlated with contaminant type and load (in sediment, water, and tissues). Biomarker analyses of both field and laboratory samples, together with correlation with contaminant levels in sediment, water, and tissue should enable us to begin unraveling the complex interactions involved with contaminant exposure and deleterious health effect in splittail.

3.2 Expected results and benefits

We anticipate biomarker expression in splittail from field and laboratory studies will correlate with changes at the ecological level (DFG and IEP ongoing studies), and that extensive water, tissue, and sediment analyses for contaminants (SFEI and USGS ongoing studies) will validate the linkage between contaminant exposure and the health of splittail population, and will pioneer an approach that can generally be applied to other aquatic ecosystems.

Cytochrome P450-dependent monooxygenases are involved in the biotransformation of organic chemicals such as coplanar PCBs (Safe, 1994.), PAHs and some pesticides (Munkitterick *et al.*, 1995). P450 enzymes like CYP1A1 initiate a catalytic cycle that leads to hydroxylation of the xenobiotic compound. The hydroxylated compound is then acted upon by phase II enzymes, transforming the initially lipophilic substance into a polar, water soluble product, which can be excreted by the organism (Goksoyr and Forlin, 1992). Induction of CYP1A1 by xenobiotic compounds has been well documented (Huggett *et al.*, 1992). Koivusaari *et al.* (1984) have shown that the P450 activities were the lowest just prior to spawning which may reflect a form of downregulation to avoid metabolism of sex steroids. The decrease in P450 suggests the fish would be least able to handle xenobiotics during spawning. Therefore, detoxification enzymes are good indicators of contaminant exposure.

Condition indices are broad measures of general health. Changes in CI specifically reflect alterations in growth and nutritional status, while fluctuation in GSI are associated with sexual maturity and reproductive status. Differences in LSI may reflect sex, sexual maturity, or general health and nutrition. Contaminant-induced alterations of somatic and/or gonadal growth will therefore be reflected by changes in these indices.

Histopathologic biomarkers are lesions in cells, tissues, or organs caused by exposure to infectious or toxic agents (Hinton *et al.*, 1992; Teh *et al.* 1997; 1999a). Histopathology has been used

in fish for the assessment of contaminant-mediated adverse effects (Adams *et al.*, 1989; Hinton *et al.*, 1992; Teh *et al.*, 1997). The advantages of histopathology is further enhanced by the incorporation of immunohistochemical localization of metallothionein, P450, and stress proteins in tissue sections (Teh *et al.* 1999a; 1999b). Histologic lesions can be examined in a tissue specific manner, allowing identification of target organs for specific xenobiotic compounds (Wester and Canton, 1986). Histologic damage in reproductive organs can be directly linked to population level effects, and detection of lesions in early life stages (embryos and larvae) of fish and other aquatic organisms is considered one of the most sensitive means of assessing adverse effects induced by xenobiotic compounds (Weis and Weis, 1987). Histopathologic analysis is particularly relevant to field investigations (Meyers *et al.* 1985; 1994), allowing rapid detection of *in vivo* toxicity, thereby helping to prioritize sites for more detailed analysis. As such, they are accurate indicators of contaminant-mediated effects at a particular site. Direct histological damage of the liver and gonads has been documented in several studies involving heavy metals, pesticides, and pulp and paper mills effluents (Singh *et al.*, 1993, 1994; Teh *et al.*, 1997; 1999b). Other than seeing direct damage such as atresia of oocytes, necrosis of spermatogonia, and intersex in male and female fish, it is often difficult to prove to what extent the damage affects such functions as enzymes and hormonal imbalances in response to contaminants or endocrine-modulating compounds. Therefore, GSI and LSI indices when coupled to histopathologic analysis of the liver and gonads, and effects of contaminants may be detected and quantified.

Results from this study also have practical application, as laboratory data linking specific contaminants with adverse effects (as well as field data correlating biomarker expression with contaminant loads) could help guide management decisions with respect to determining acceptable contaminant levels in the environment. Finally, if assays are refined, many of the proposed biomarkers could potentially be used to monitor contaminant exposure and effect in organisms from a variety of contaminated aquatic habitats, and help evaluate progress of remediation efforts.

3.3 Linkages and System-Wide Ecosystem Benefits

Currently, there is no funded project addressing chronic toxicity of environmental contaminants in splittail. Several projects which provide valuable information on water, tissues and sediments contaminant analyses have been funded by CALFED and are readily available on the USGS and SFEI web sites. CALFED has also funded "Assessment of Ecological and Human Health Impacts of Mercury in the Bay-Delta Watershed" that includes monitoring of mercury in fish tissue. Dr. Davis and Mr. Ichikawa are the principal investigators for the fish component of that work, and will ensure that the proposed work is closely coordinated with the designated action. We propose a 3-year extensive field and laboratory investigation of all life stages (embryos, larvae, juveniles, adults) of splittail where four functional categories of biological indicators will be measured. The main product of this study will be a proven cost-effective technique or approach that the CALFED can use to (a) address splittail population decline (Pp 24-25 ERP V-II), (b) assess specific indicators approach and strategic objective (Pp 6, 32, 40, and 421 ERP V-I), (c) determine the environmental effects of Se, Hg, and pesticides (ERP Section 5. Water quality program), and (d) implement effective state-of-the-art environmental management strategies and regulatory compliance issues (Pp 1-4 of ERP V-I and II). Since the contaminants of concern (i.e., Se, Hg, Diazinon, and PCBs, etc.) are expected to be similar at most sites in California watersheds, the types of environmental effects are also expected to be similar if the contaminants were found. Thus, the approach that will be demonstrated through this project should be generally applicable at most sites where contamination and related environmental compliance and regulatory issues are of a concern to the CALFED.

4. Technical Feasibility and Timing

It is apparent that while contaminant analysis of water and sediments is a useful means by which risks to aquatic organisms are assessed, chemical approaches alone (including discovery of contaminants in the tissue of affected species) are insufficient to fully predict the biological impacts of exposure (Iannuzzi *et al.*, 1995). Many chemicals measured in sediments and water are not present in bioavailable forms (Hamelink *et al.*, 1994), hence risk estimates may be overestimated. Alternatively, information regarding the acute or chronic toxicity of a chemical or a mixture of chemicals is lacking and risks may be underestimated. Although, extensive characterization of the acute toxicity of contaminants has allowed the successful implementation of chemical discharge limitations and the protection of aquatic organisms against these contaminants (Stephen 1975). The procedure for characterization of chronic toxicity of contaminants is more complex and usually is hindered by the inability to maintain or induce reproduction under laboratory conditions, and the duration of time required to assess contaminant toxicity over the full life cycle of the organism. To circumvent such difficulties, we chose splittail as our sentinel fish model for field and laboratory chronic toxicity evaluations. Splittail was selected as a suitable target species. Like many other native fish species, its population in California has declined drastically over the past 5 decades. Unlike other native fish species where laboratory culture is difficult and expensive, splittail is easily cultured at low cost and will spawn in captivity providing access to all life stages. Therefore, fish can be exposed at different life-stages in the laboratory and maintained for one or two generations to assess the chronic toxicity effects of contaminants. Biomarker expressions of field and laboratory fish can be compared. Growth and reproduction impairments can be assessed in laboratory study to verify the field findings.

For several years, splittail have been successful spawn in the Aquaculture & Fisheries Program at UCD. Therefore, laboratory-raised splittail, tanks and equipments are available when needed for the initiation of contaminant exposure. In addition, DFG splittail population monitoring project funded by the IEP will sampling splittail at various location in the Sacramento-San Joaquin, and Bay-Delta regions in the late fall and winter. SFEI (RMP, SRWP, CISNET) projects will provide additional information on concentrations of these contaminants in water, sediment, and fish tissue at the Petaluma River, Napa River, Suisun Bay, and Sacramento River locations. Furthermore, the information on contaminant monitoring data from projects (SFEI and USGS) already funded by CALFED, can be easily assess through the web sites. Therefore, we are confident that all 3 tasks mention in our scope of work can be fulfilled.

4.1. Fish Collections and Time of Sampling:

Adult and juvenile splittails will be collected between June and September. The timing of the collection is such that it will not present a hazard to winter-run chinook salmon. 150' research gill nets set for 15-20 min will be used to capture adults, 100'x6' beach seine with 1/4" mesh set by boat to capture juveniles, both gears have been used successfully by CDFG/Interagency Ecological Program to capture splittail. Fish collecting during the spawning season will be by hook-and-line when necessary to avoid impacting endangered salmonids.

4.2. Biomarkers approach:

The biomarker approach is not only a proven technique for assessing environmental status due to contamination, but also provides biological relevant endpoints that can be used to provide more accurate and reliable ecological risk assessments (Teh *et al.* 1997, Adam *et al.* 1999). This is a proven technique which does not require further research and development prior to field demonstration and capable of full scale implementation.

5. Monitoring and Data Collection Methodology

5.1. Biological/Ecological Objectives:

The goal of the proposed research is to combine ongoing efforts by DFG and IEP in assessing the splittail population, USGS and SFEI data on contaminant levels in water, sediment, and tissue samples, and the field and laboratory integrated biomarker approach (Table 1) to establish a causative link between contaminant exposure and chronic deleterious health effect in splittail. We anticipate that the laboratory exposure will correlate with the field exposure and thus allowing us to identify selected biomarkers that are critical for the health of the splittail.

5.2. Monitoring Parameters and data collection Approach: Before studies are initiated, detailed protocols will be developed. Methods used in subsequent portions of the project will be developed into standard operating procedures (SOPs).

5.2.1. Field and Laboratory Study: We will collect 30 adults and 30 juveniles from three sites (total 90 adults and 90 juveniles) using short gill net sets, beach seines or hook and line. A maximum of 3 days of collection effort per site will be made between June and October of each year. Laboratory exposures will use representatives of major contaminant groups determined in field study. 1,080 embryos, three replicates of 60 per replicate (5 chemicals and one control) will be bath exposed to Se, Hg, Se/Hg, diazinon, permethrin, and control water for 7 days (d) or until hatched. Three replicates of 30 Juvenile and 30 adult per replicate (540 juveniles and 540 adults) will be exposed to Se, Hg, Se/Hg, diazinon, permethrin, and control water. Exposure routes will be diet for metals (30 d) or aqueous for pesticides (7 d).

5.2.2 Chemical Analyses: Chemical analyses will be performed by laboratories under subcontract with SFEI: the CDFG Water Pollution Control Laboratory (trace organics) and the CDFG Moss Landing Marine Laboratory (trace elements). The fish tissue samples will be analyzed for mercury, selenium, diazinon, organochlorine pesticides, and PCBs.

5.2.3 Data Evaluation Approach: Data analyses will evaluate and integrate metal, pesticides and organochlorine concentrations in body and gonadal tissue to condition indices (CI), developmental toxicity, growth, fertility, fecundity, biochemical and histopathological results to evaluate whether contaminant concentrations in various matrices can be correlated with evidence of splittail population declines. Biomarkers selected for this study are those we have experience with and which have proven track records with regard to use in study of chronic toxicity.

Data will be entered into a spreadsheet program (Microsoft Excel) and sorted on the basis of gender, organ, and treatment group. Average scores for each lesion will be compared using Mann-Whitney Rank Sum Test between sites and experiments. ANOVA will be used to test site and laboratory exposure effects. Existing contaminant-related literature pertaining to our splittail project will be reviewed. In the final year, correlation of the four functional categories of biological indicators, DFG splittail population monitoring data, and SFEI/USGS spatial patterns of contaminant concentrations in tissue, water, and sediments will be integrated using multivariate statistical analysis to determine relationship between the contaminant loading in the environment and fish health. Data for acceptability and scientific quality will be reviewed. Quarterly and final report in hardcopy and electronic format that details study in progress, and contains a compilation of all raw data record will be submitted to CALFED. Manuscripts from the proposed research will be submitted to a high quality scientific journals for peer review and publication. Results will be disseminated widely through participation in workshops and seminars, and presentation of papers at an international and national meeting.

Table 2. Monitoring and Data Collection Information

Biological/Ecological Objectives: Chronic Toxicity of Environmental Contaminants in Sacramento Splittail (<i>Pogonichthys macrolepidotus</i>)			
Splittail	Data Collection	Data Evaluation	Comments/Data Priority
TASK 1-1A. Field evaluation of contaminant exposure (90 adults)	<ol style="list-style-type: none"> 30 per site for 3 sites will be collected using short gill net sets, beach seines or hook and line between June and September Transport 30 (10 per site) to UCD, rear fish until spawn Collect 5 fishes per site for body burden analysis. 	<ol style="list-style-type: none"> Indicators of contaminant exposure (IND-1) Indicators of fish condition indices (IND-2) Indicators of organs and reproductive dysfunctions (IND-3) Indicators of individual level responses. (IND-4) 	<ol style="list-style-type: none"> Determine body burdens Determine detoxification enzymes induction related to contaminant exposure Determine vitellogenin induction in male fish for endocrine disruptors screening. Gross morphological abnormalities examinations for immune response screening Immunohistochemical and histopathological analyses for rapid detection of in vivo toxicity, helping to prioritize sites for more detailed analysis. Direct liver and gonadal analysis to provide data of reproductive impairments. Determine condition indices to measure adverse health effects of contaminants. Determine reproductive success using fecundity and fertility assays.
TASK 1-1B. Field evaluation of contaminant exposure (90 juveniles)	30 per site for three sites will be collected using short gill net sets or beach seines between June and September	<ol style="list-style-type: none"> IND-1 IND-2 IND-3 	1. Item 1A2, 1A4, 1A5 and 1A 6 will be assessed.
TASK 2-2A. Laboratory evaluation of contaminant exposure (540 Juvenile/ 540 adult)	<ol style="list-style-type: none"> Three replicates of 30 Juvenile and 30 adult per replicate will be exposed to Se, Hg, Se/Hg, diazinon, permethrin, and control water. Exposure routes will be dietary for metals (30 d) or aqueous for pesticides (7 d). Sample fish at day 0, 90, and 180 after exposure. Collect 5 fishes for body burden analysis. 	<ol style="list-style-type: none"> IND-1 IND-2 IND-3 IND-4 	<ol style="list-style-type: none"> Item 1A1to 1A7 will be assessed. Determine the growth and chronic effect of contaminant exposure as a function of time before and after exposure

<p>TASK 2-2B. Laboratory evaluation of contaminant exposure (1080 embryos)</p>	<p>Three replicates of 60 embryos per replicate will be bath exposed to Se, Hg, Se/Hg, diazinon, permethrin, and control water for 7 days (d) or until hatched.</p>	<p>1. IND-1 2. IND-2 3. IND-3</p>	<p>1. Item 1A1 to 1A6 will be assessed 2. Using gross and histological analyses to determine developmental abnormalities in embryos and larvae (hatchling) 3. Determine the growth and chronic effect of contaminant exposure as a function of time before and after exposure</p>
<p>TASK 3 Field and laboratory evaluations</p>	<p>1. Data will be entered into a spreadsheet program (Microsoft Excel) and sorted on the basis of gender, organ, and groups.</p>	<p>1. Average scores for each lesion will be compared using Mann-Whitney Rank Sum Test between sites and experiments. 2. Analysis of variance (ANOVA) will be used to test site and laboratory exposure effects.</p>	<p>1. Submit quarterly and final report in hardcopy and electronic format that details study in progress, and contains a compilation of all raw data recorded to CALFED. 2. Review existing contaminant-related literature pertaining to our splittail project. 3. Review data for acceptability and scientific quality.</p>
	<p>Compile and integrate the four functional categories of biological indicators, DFG splittail population monitoring data, and SFEI/USGS spatial patterns of contaminant concentrations in tissue, water, and sediments</p>	<p>using multivariate statistical analysis to determine relationship between the chronic toxicity effects of contaminant and fish health (SYSTAT 8.0)</p>	<p>Manuscripts from the proposed research will be submitted to a high quality scientific journals for peer review and publication. Results will be disseminated widely through participation in workshops and seminars, and presentation of papers at an international and national meeting.</p>

6. Local Involvement

This is a research project where 80% of the work will be done within a research laboratory. As such, local, environmental, landowner, conservancies and CRMPS, groups were not aware of this project.

This project, in conjunction with ongoing biomonitoring efforts of fish population by DFG and water, sediment, and tissue contaminant monitoring by SFEI and USGS, to evaluate the chronic effects of contaminants on the health of splittail under laboratory and field conditions will provide valuable information for future environmental compliance and regulatory studies and the ecological risk assessment process. These studies also have practical application as laboratory data linking specific contaminants with adverse chronic effects (as well as field data correlating biomarker expression with contaminant exposure) could help guide management decisions with respect to determining acceptable contaminant levels in the environment.

Manuscripts from the proposed research will be submitted to a high quality scientific journals for peer review and publication. Results will be disseminated widely through participation in workshops and seminars, and presentation of papers at an international and national meeting.

This project support ongoing efforts by the USFW Service and the IEP in recovering threatened and endangered fish populations in the Sacramento-San Joaquin system by facilitating restoration planning and monitoring. We will be working in close collaboration with DFG's splittail monitoring survey, IEP funded splittail culturing projects, and various water quality monitoring programs (e.g. DFG, SWRCB, SFEI, USGS)

We do not foresee any third party impacts to result from this study.

7. Cost

Three years of support are requested. Project schedule is shown in Table 3. Budget costs are broken down by Principal Investigator and year (Table 4). The budget for each task on a quarterly basis is shown in Table 5.

Dr. Silas Hung will serve as Lead Principal Investigator. Direct salary costs for Dr. Hung represent a 1728-hr commitment to the project. Specific tasks include overall organization of the project and coordination with Drs. Teh and Davis, oversee the study and assist with experimental design and interpretation of data, laboratory exposure/growth work, enzymes and vitellogenin analyses, presentations, and report writing (Tasks 1,2,3 Table 2). Dr. Hung requires two assistants, a 2880-hr commitment of a Laboratory Assistant III (TBA) for histological processing, as well as a 2880-hr commitment of Post-Graduate Researcher (D. Deng) who specializes in fish husbandry and nutrition, and will be responsible for preparation of contaminant-laden test diets. Three student assistants (8640-hr) will also be hired to assist in maintaining the splittail colony. Miscellaneous Costs include supply funds for histology, immunohistochemistry, enzymes and vitellogenin analyses, laboratory exposures, computer software, and general laboratory/office operation related to the project. In addition, these costs include travel funds for research, project meetings, and presentations at the national conference of a professional society.

Dr. Swee Teh (Co-Principal Investigator) will be responsible for all histopathological analyses and laboratory exposures. Direct labor hours reflect a 2100-hr commitment of Dr. Teh on these and his primary task of evaluating and interpreting the large number of histopathological preparations (several thousand slides) (Tasks 1,2, Table 2). Miscellaneous Costs consist of supply funds for computer software, and general office operation, and travel to project meetings and to present findings at one national conference of a professional society.

Dr. Jay Davis (Co-Principal Investigator) will be responsible for all chemical analyses, field collections, and interpretation of these results (Tasks 1-1A, 2-2A, Table 2). Direct Salary Costs for Dr. Davis include 300-hr commitment to the project, as well as a 60-hr commitment of A. Yang, for assistance in the contaminant analysis. Chemical analyses and field sampling will be performed by laboratories under subcontract with SFEI (Dr. Davis): the CDFG Water Pollution Control Laboratory (trace organics) and the CDFG Moss Landing Marine Laboratory (trace elements). The fish tissue samples will be analyzed for mercury, selenium, diazinon, organochlorine pesticides, and PCBs.

Schedule.

1. Diet preparation, laboratory exposures and maintenance, tissues sampling:
Drs. Hung, and Teh, postgraduate researcher, 3 student assistants.
2. Biochemical biomarkers (detoxification enzymes and vitellogenin):
Drs. Hung and Teh, Postgraduate researcher
3. Histopathological biomarkers (histological, enzyme- and immunohistochemical endpoints):
Dr. Teh and histotechnician
4. Field sampling: Dr Davis, Gary Ichikawa (DFG), A Yang.
5. Contaminant analyses: SFEI and SDFG Water Pollution Control Laboratory
6. Data analyses, report and manuscript preparation: UC statistician, All principal and co-principal investigators

Table 3. Project Schedule

Task and Milestones	Oct-Dec 1999	2000	2001	Jan-Sept (2002)
Task 1 and 2. Chemical analysis of tissue (Davis)		[-----]	[-----]	[-----]
Task 1. Field sampling (Davis, Ishikawa)		[---]	[---]	[---]
Task 1 and 2. Field indicators study (Teh, Hung)		[---]	[---] [---]	[-----]
Task 1 and 2. Laboratory indicators study (Hung, Teh)	[---]	[-----]	[-----]	[-----]
Task 3. Raw data, statistic analysis, quarterly, annual, and final report. (Teh, Hung, and Davis)		[-----]	[-----]	[-----]

Table 4. Project Cost Summary by task

TASK	Direct Labor Hours	Direct Salary and Benefits	Service Contracts	Material and Acquisition Costs	Miscellaneous and other Direct Costs	Overhead and Indirect Costs	Total Cost
Task 1 and 2 Diet preparation, laboratory exposures and maintenance, tissues sampling, bichemical biomarkers (Dr. Hung (PI), TBA, D. Deng, 3 Students assistants (SA)	1728 (Hung)	In-Kind	0	0	\$60,000	6,000	\$66,000+ In kind
	2880 (TBA)	\$63,750	0	0	0	6,375	\$70,125
	2880 (Deng)	\$57,600 Inc. fees	0	0	0	5,760	\$63,360
	8640 (3 SA)	\$60,480	0	0	0	\$6,048	\$66,528
Task 1 and 2 Histopathologic biomarker approach include gross and microscopic analysis of liver and gonads, field dissection of adult fish, gross and microscopic analysis of embryo developmental abnormalities Dr. Teh (co-PI)	2,100 (Teh)	\$157,500	0	0	\$18,000	0	175,500

Task 1 and 2 contaminant analyses, Field Sampling, (Drs. Davis and Teh (Co- PI), A. Yang, G. Ichikawa, and CDFG Laboratory)	300 (Davis),	\$15,194	\$144,594 (CDFG)	0	0	\$15,755 (Davis + CDFG)	\$175,543
	60 (Yang)	\$1,698	\$42,300 (Ichikawa)	0	0	\$630 (Yang)	\$44,628
	200 (Teh)	In-Kind	0	0	0	0	In-Kind
Task 3 (Drs. Hung, Teh, and Davis. Submit quarterly and final report in hardcopy and electronic format that details study in progress, and contains a compilation of all raw data recorded to CALFED. Manuscripts from the proposed research will be submitted to a high quality scientific journals for peer review and publication.	120 (Hung)	0	0	0	0	\$3,000 (Publicati on cost)	\$3,000
	120 (Teh)	\$9,000	0	0	0	0	\$9000
	120 (Davis)	In-Kind	0	0	0	0	In-Kind
Total costs for 3 years				\$673,684.00			

Table 5. Quarterly budget

TASK	Task 1 Hung	Task 2 Hung	Task 3 Hung	Task 1 Teh	Task 2 Teh	Task 3 Teh	Task 1 Davis	Task 2 Davis	Task 3 Davis	Total Budget
Quarterly Budget Oct-Dec 99	0	22,168	\$0	0	3,000	750	0	0	0	25,918
Quarterly Budget Jan-Mar 00	0	22,168	\$0	0	6,000	750	0	0	0	28,918

Quarterly Budget Apr-Jun 00	0	22,168	\$0	7500	10,000	750	0	0	0	40,418
Quarterly Budget July-Sep 00	10,000	12168	\$0	7,500	10,000	750	43,745	29,645	0	113,808
Quarterly Budget Oct-Dec 00	22,168	0	1000	7,500	3,000	750	0	0	0	34,418
Quarterly Budget Jan-Mar 01	0	22,168	0	0	16,000	750	0	0	0	38,918
Quarterly Budget Apr-Jun 01	0	22,168	0	0	17,500	750	0	0	0	40,418
Quarterly Budget Jul-Sep 01	10,000	12,168	1,000	7,500	10,000	750	43,745	29,645	0	114,808
Quarterly Budget Oct-Dec 01	22,168	0	0	7,500	3,000	750	0	0	0	33,418
Quarterly Budget Jan-Mar 02	0	22,168	0	0	13,000	750	0	0	0	35,918
Quarterly Budget Apr-Jun 02	0	22,168	0	7,500	16,000	750	0	0	0	46,418
Quarterly Budget Jul-Sep 02	10,000	12,165	1,000	7,500	15,500	750	43,745	29,646	0	120306
Total	74,336	19,167 7	3,000	52,50 0	12,300 0	9000	131235	88,936	0	673,684

8. Cost-Sharing

This is a new project determining chronic toxicity of splittail. Therefore, there is no cost-sharing in this project. However, the main product of this study will be a proven cost-effective technique or approach that the CALFED can use to (a) address splittail population decline (Pp 24-25 ERP V-II) (b) assess specific indicators approach and strategic objective (Pp 6, 32, 40, and 421 ERP V-I) (c) determine the environmental effects of Se, Hg, and pesticides (ERP Section 5. Water quality program), and (d) implement effective state-of-the-art environmental management strategies and regulatory compliance issues (Pp 1-4 of ERP V-I and II). Since the contaminants of concern (i.e., Se, Hg, Diazinon, and PCBs, etc.) are expected to be similar at most sites in California watersheds, the types of environmental effects are also expected to be similar if the contaminants were found. Thus, the approach that will be demonstrated through this project should be generally applicable at most sites where contamination and related environmental compliance and regulatory issues are of a concern to the CALFED. These studies also have practical application as laboratory data linking specific contaminants with adverse chronic effects (as well as field data correlating biomarker expression with contaminant exposure) could help guide management decisions with respect to determining acceptable contaminant levels in the environment.

All data from this study will be published or be available to interest parties. We will share the results of this study by creating a special chronic toxicity website or created an attachment of the results to IEP, SFEI, USGS Websites.

In-kind contributions were made by Drs Hung (1728-hr), Teh (200-hr) and Davis (120-hr) to minimize the cost of this project (Table 4).

9. Applicant Qualifications

Dr. Silas Hung, a professor of the Department of Anima Science, will serve as lead Principle Investigator and main contact with CALFED for this project. Dr. Hung, has 20 years of continuous experience in fish rearing, nutrition, and biochemistry. He is the PI of an ongoing investigation on the Selenium requirement of white sturgeon and is collaborating with Dr. Teh to investigate the Se toxicity in white Sturgeon. Dr. Hung will have overall responsibility for the project and for all progress reports. He also is the PI in many studies on the feeding, nutrient requirements and utilization of white sturgeon and rainbow trout.

Dr. Swee J. Teh is the owner of Toxicopathology Consulting, an independent consulting firm with extensive capabilities in histopathology. He has 12 years of extensive field and laboratory research experience on ecotoxicology and biomarker study. He has also experienced in projects and experimental design and managing contracts and grants' total >\$1 millions per year. Dr. Teh is a comparative pathologist with extensive field and laboratory research experience. He will be primarily responsible for the histopathological assessment of splittail obtained from the field, the corresponding histopathological assessment of control fish exposed to chemical mixtures in the laboratory, the interpretation and integration of this data, and the submission of quarterly and annual reports with Drs. Hung and Davis. All personal equipments (computer and microscope) are on hand. He has been a Principle Investigator and/or managed grants from various Federal agencies, including USEPA (totalling over \$1 million), NCI (totalling over \$2.4 million), and USDE (\$275K). Dr. Teh has extensive experience with enzyme- and immunohistochemistry, light and electron microscopy, fish pathology, carcinogenesis, and morphometry. He is the primary or co-author author on over a dozen referred publications related to fish histopathology and immunohistochemistry, including Teh and Hinton (1993), Teh et al. (1997), and Teh and Hinton, (1998).

Dr. Jay A. Davis received his Ph.D. in Ecology at the University of California, Davis in 1997. Dr. Davis has worked on contaminant issues in the San Francisco Estuary since 1986. Dr. Davis worked for the Aquatic Habitat Institute from 1986 to 1992. During this period he co-authored several Institute reports, including Status and Trends Reports on Pollutants and Dredging and Waterway Modification for the San Francisco Estuary Project. He joined the staff of SFEI in 1995. Dr. Davis is part of a team that manages the Regional Monitoring Program for the San Francisco Estuary, and is currently managing three projects examining contaminants of human health concern in fish from the Bay, the Sacramento River watershed, and the Delta, and two projects examining the ecological significance of accumulation of persistent chemicals in fish in the Bay and Delta.

10. Compliance with Standard Terms and Conditions

The financially responsible applicant is a full-time professor of the University of California-Davis, Davis California, 95616. It is the applicant's understanding from Attachment D, table D-1 of the RFP that no supplemental forms are required until at the time of final contract.

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