99D-115

4.5 PSP Cover Sheet (Attach to the front of each proposal)

Proposal Title: A New Approach to Assess the Effect of Ecosystem Restoration Efforts on Contaminant Bioavailability

Applicant Name: Donald P. Weston, Ph.D. THE REGENTS OF THE UNIVERSITY OF CALIFORNIA

Mailing Address: Univ. of Calif., Berkeley, 3060 VLSB, Berkeley, CA 94720-3140

Telephone: 510-231-5626

Fax: 510-643-6264

Email: dweston@uclink4.berkeley.edu

Amount of funding requested: $ 631,939 (State) 711,773 (Fed.) for 3 years

Indicate the Topic for which you are applying (check only one box).

- ☐ Fish Passage/Fish Screens
- ☐ Habitat Restoration
- ☐ Local Watershed Stewardship
- ☒ Water Quality
- ☐ Introduced Species
- ☐ Fish Management/Hatchery
- ☐ Environmental Education

Does the proposal address a specified Focused Action? ____ yes __X__ no

What county or counties is the project located in? Madera, Contra Costa, Solano, San Joaquin, Yolo

Indicate the geographic area of your proposal (check only one box):

- ☐ Sacramento River Mainstem
- ☐ Sacramento Trib: _____
- ☐ San Joaquin River Mainstem
- ☐ San Joaquin Trib: _____
- ☐ Delta: _____
- ☐ East Side Trib: _____
- ☐ Suisun Marsh and Bay
- ☐ North Bay/South Bay: _____
- ☒ Landscape (entire Bay-Delta watershed)
- ☐ Other: _____

Indicate the primary species which the proposal addresses (check all that apply):

- ☐ San Joaquin and East-side Delta tributaries fall-run chinook salmon
- ☐ Winter-run chinook salmon
- ☐ Late-fall run chinook salmon
- ☐ Delta smelt
- ☒ Splittail
- ☒ Green sturgeon
- ☐ Migratory birds
- ☒ Other: All bottom-feeding fishes
- ☐ Spring-run chinook salmon
- ☐ Fall-run chinook salmon
- ☐ Longfin smelt
- ☐ Steelhead trout
- ☐ Striped bass
- ☐ All chinook species
- ☐ All anadromous salmonids

Specify the ERP strategic objective and target (s) that the project addresses. Include page numbers from January 1999 version of ERP Volume I and II:

Contaminants (p. 506); Green sturgeon (p. 205); White sturgeon (p. 402)

Splittail (p. 209)

Indicate the type of applicant (check only one box):

☐	State agency	☐	Federal agency
☐	Public/Non-profit joint venture	☐	Non-profit
☐	Local government/district	☐	Private party
☒	University	☐	Other: _____

Indicate the type of project (check only one box):

☐	Planning	☐	Implementation
☐	Monitoring	☐	Education
☒	Research		

By signing below, the applicant declares the following:

1.) The truthfulness of all representations in their proposal;

2.) The individual signing the form is entitled to submit the application on behalf of the applicant (if the applicant is an entity or organization); and

3.) The person submitting the application has read and understood the conflict of interest and confidentiality discussion in the PSP (Section 2.4) and waives any and all rights to privacy and confidentiality of the proposal on behalf of the applicant, to the extent as provided in the Section.

_____ Approved for the University of California at Berkeley

Printed name of applicant

Lynn E. Deatz
Senior Research Administrator
Sponsored Projects Office, 336 Sproul Hall
Berkeley, CA 94720-5940
(510) 643-6113 FAX: (510) 642-6236

Signature of applicant

A NEW APPROACH TO ASSESS THE EFFECT OF ECOSYSTEM RESTORATION EFFORTS ON CONTAMINANT BIOAVAILABILITY

Principal Investigators:

DR. DONALD P. WESTON (primary contact), Associate Adjunct Professor
Univ. of California Berkeley, 1301 S. 46th St., Bldg.112, Richmond, CA 94804
510-231-5626; Fax: 510-643-6264; dweston@uclink4.berkeley.edu

DR. ROBERT P. MASON, Assistant Professor
Chesapeake Biological Laboratory, Univ. of Maryland, Solomons, MD 20688
410-326-7387; Fax: 410-326-734; mason@cbl.umces.edu

DR. LAWRENCE M. MAYER, Professor
Darling Marine Center, University of Maine, Walpole ME 04573
207-563-3146, ext. 237; Fax 207-563-3119; lmayer@maine.maine.edu

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I-018943

EXECUTIVE SUMMARY

Ecosystem restoration efforts often modify the physical and chemical conditions in an environment so as to alter the bioavailability of contaminants in the system. Flooding of agricultural lands to create wetland habitat can remobilize soil-bound pesticides or heavy metals. Changes in the input of organic matter, as might accompany water flow or land use changes through ecosystem restoration, would alter the bioavailability of contaminants capable of chemically interacting with organic carbon. For example, bioavailability of methylmercury is highly dependent upon the concentration of organic carbon, and restoration efforts that reduce organic input could have the unintended consequence of increasing mercury bioavailability. In countless restoration scenarios, there is a need to quantify contaminant bioavailability and the effect of habitat modifications on this parameter in order to protect the very species on which restoration activities are focused.

We propose a novel approach to measure the bioavailability of sediment-associated contaminants that can be used in support of ecosystem restoration actions. It is generally accepted that chemical measures of contaminant concentration provide environmental managers little useful information on contaminant bioavailability simply because the exotic solvents used in conventional chemical analyses extract far more of the contaminant than biota are capable of accumulating. Therefore we employ digestive fluid, and ultimately a synthetic equivalent of that fluid, as a biologically relevant extractant. The sediments of concern are incubated in vitro in the fluid, and the proportion of contaminant extractable is a measure of bioavailability. Our work over the past few years has shown that the digestive fluid assay is far superior to conventional chemical analysis in predicting bioavailability, and even has significant advantages over whole animal bioaccumulation testing because of the broad range of environmental conditions over which it can be employed. The Environmental Protection Agency has funded us in a multi-year effort to develop the technique for polynuclear aromatic hydrocarbon assessment, and we have found the digestive fluid approach to give results comparable to more traditional measures of bioavailability. The U.S. Navy has supported the work for assessment purposes at naval bases with a wide range of organic and heavy metal contaminants, and in these tests the digestive fluid approach proved an excellent predictor of heavy metal bioavailability to a clam. Finally, we have used the technique to measure the bioavailability of methylmercury to amphipods, and found a high degree of correlation between methylmercury extraction by digestive fluid and bioaccumulation by amphipods. All these recent studies have demonstrated that the technique can provide a measure of bioavailability with many significant advantages over current techniques, and that it is applicable to the types of contaminants of concern to CALFED and its member agencies.

We propose to demonstrate the utility of this technique in quantifying the bioavailability of sediment-associated mercury and selenium, two elements of considerable concern in the San Francisco Bay Delta and its tributaries. The digestive fluid we will be using in the initial tasks is from the white sturgeon. This species was chosen because of the availability of farmed animals, but sturgeon in general are known to ingest substantial amounts of sediment during feeding, are suspected to be at risk in San Francisco Bay because of high tissue selenium levels, and are of general concern because of the recreational fishing in the Bay-Delta which it supports. Our studies, however, are not sturgeon-limited, for we have substantial data that will allow extrapolation to other species, and ultimately we will develop a synthetic digestive fluid for use in sediment assessment that will be modelled after fluids from multiple sediment-ingesting species.

We propose a two year laboratory effort to develop the approach for assessment of mercury and selenium bioavailability, followed by a field demonstration in the third year in collaboration with on-going CALFED-supported studies where bioavailability questions are critical.

- Task 1 - We will establish the extent of mercury and selenium desorption from sediments when those sediments are incubated in digestive fluid, and determine which sedimentary variables such as organic content control the extent of solubilization. This work will lead to an understanding of the environmental factors influencing mercury and selenium bioavailability, and how this bioavailability depends upon the chemical species in which the element is found.

We will also establish the range of bioavailability typically encountered in the San Francisco Bay watershed. Our past work with the technique has demonstrated that only a small fraction of the mercury and selenium which are chemically quantifiable is actually available to biota (i.e., < 10% of the inorganic mercury, <50% of the organic mercury, and <20% of the inorganic selenium). We will also evaluate the utility of the in vitro extraction in mimicking in vivo digestive solubilization of contaminants.

- Task 2 - Bioaccumulation is a two-step process involving digestive solubilization followed by absorption of the contaminant across the gut wall. The digestive solubilization technique incorporates only the first step, thus it is necessary to explictly test the presumption that all or nearly all the solubilized contaminant is subsequently absorbed. Work to date with other contaminants suggests that solubilization is the indeed the rate limiting step, but we need to explicitly test this hypothesis for mercury and selenium. Digestive absorption of these substances (both organic and inorganic forms) will be measured in both whole sturgeon and also in perfused gut preparations. We will also examine how mercury and selenium competitively interact with one another in solubilization and absorption processes.

- Task 3 - Use of sturgeon digestive fluid for contaminant extraction is an essential initial step, but broad adoption of a digestive fluid approach to measuring bioavailabilty will require a more readily available surrogate. For example, work in our lab has shown that commercially-available bovine serum albumin is an excellent mimic of invertebrate digestive fluid in estimating copper bioavailability. In Task 3 we will initially study the mechanisms underlying digestive solubilization of mercury and selenium. The results from the solubilization mechanism work will enable us to design synthetic cocktails that contain the relevant solubilizing agents. These agents might take the form of enzymes, inert proteins, or other chemicals/biochemicals found to play a role in gut dissolution of mercury and selenium.

- Task 4 - CALFED is currently funding a study of mercury in restored wetlands, and an important component of this project is how time since flooding and other environmental conditions affect bioavailability of sedimentary mercury. In the on-going CALFED study bioavailability is being quantified by bioaccumulation in resident fish and invertebrates. In Task 4 we will assess mercury bioavailability by in vitro digestive fluid extraction, using both sturgeon digestive fluid and the synthetic mimic, and compare results to the resident organism bioaccumulation data. The intent of Task 4 and Task 5 below is to demonstrate application of the digestive fluid technology in an environmental restoration context.

- Task 5 - CALFED is currently funding development of an Algal-Bacteria Selenium Removal (ABSR) system to remove selenium from agricultural drainwater. The selenium removed from these wastewaters accumulates in sludges at the bottom of anaerobic ponds, and ultimately there will be a need to dispose of these selenium-rich sludges. Digestive fluid extraction techniques provide a means to assess the bioavailability of selenium from this material in order to guide sludge management decisions. Of particular interest is how transfer of the sludges from their current anaerobic environment to an aerobic environment might affect selenium bioavailability, and we will assess this with both sturgeon and synthetic digestive fluids.

All three co-investigators have worked and published extensively on this new technology for assessment of contaminant bioavailability. Its utility has been demonstrated for other contaminants, and we believe it has great potential for mercury and selenium as well. It could, in fact, be applied to any other sediment-bound contaminant including hydrophobic pesticides and other trace metals. We believe it offers some significant advantages to other measures of bioavailability being used by CALFED and many other resource management agencies, and the proposed laboratory development followed by field demonstration of its merits should provide the basis for integration of the approach into environmental restoration.

PROJECT DESCRIPTION

Assessing contaminant bioavailability is important to measuring the success of restoration and avoid harming the very species which the restoration is intended to enhance. Assessments of environmental quality have generally relied on chemical characterization of contaminant concentration or biological measures of bioavailability, but both approaches have significant shortcomings (discussed below). We have been developing a technique to measure bioavailability of particle-bound contaminants by incubating the sediment in vitro with the digestive fluid of deposit-feeding invertebrates and measuring the amount of contaminant that is solubilized in the fluid. The approach mimics the digestive environment that a contaminated particle would encounter as it transits an organism's gastrointestinal tract. Our work has shown that the bioavailable fraction is almost always less than half of the contaminant measurable by conventional chemical means, and in many instances may be only a few percent of the chemically quantifiable contaminant. The approach has been used to make contributions in understanding bioavailability of PAH and certain metals (Table 1), and we have shown the technique to be an excellent predictor of mercury uptake by a benthic invertebrate (Lawrence et al., in press).

We propose to develop the digestive fluid extraction technique to measure how restoration activities affect contaminant bioavailability. We will focus on mercury (Hg) and selenium (Se), two substances of concern in the Bay-Delta system and of particular interest to CALFED. It is well documented that the diet is the dominant source for bioaccumulation of both Hg and Se (Phillips and Buhler, 1978; Luoma, et al., 1992; Hall et al., 1997), and thus it is important to use a bioavailability assay based on digestive desorption of the contaminants from food particles. Our proposed research plan utilizes the digestive fluid solubilization approach to achieve the following objectives, each of which is described as a specific task below:

1) Assess the bioavailability of various organic and inorganic forms of Hg and Se in the diet with respect to their relative extents of solubilization within the gut environment; and determine the effect of sediment properties (e.g., TOC, AVS, redox status, iron content, grain size) in reducing or enhancing Hg and Se bioavailability;
2) Determine the digestive absorption of various organic and inorganic forms of Hg and Se, and establish if digestive solubilization alone is a good predictor of absorption potential;
3) Develop a synthetic extractant that simulates the chemistry of the gut environment, to estimate the bioavailability of particle-bound contaminants to demersal-feeding fishes.
4) Test and demonstrate the utility of the digestive fluid approach in two on-going CALFED-funded projects in which assessment of Hg or Se bioavailability is crucial.

Task 1 - Digestive solubilization: Given that digestion is a dominant route for Hg/Se uptake, it follows that digestive solubilization will control organismal exposure. Any predictive understanding of bioaccumulation must examine factors that control digestive solubilization. The principal tool we will use for these studies is digestive fluid solubilization in which the sediment is incubated in vitro in digestive fluid extracted from the organism, and the extent of contaminant solubilization from the matrix quantified. We will extract digestive fluid from the stomach of white sturgeon (Acipenser transmontanus), sediment and gut fluid will be incubated together, and then centrifuged to recover the supernatant which is analyzed for Hg and Se.

For some tasks we will use contaminated field-collected sediments, but in some cases we will spike sediments with radioactive ^{203}Hg or ^{75}Se. Use of the radioactive forms allows spiking at lower concentrations than would be achievable with stable isotopes, and gamma counting can be done quickly and at lower cost. We have found that spiked and in situ-contaminanted Hg do not differ in extents of desorption from sediment, but we will be aware of potential differences in experimental design.

We will begin this task with a series of incubations using sturgeon gut fluids and sediments collected from the Delta, high Se areas of the San Joaquin River and tributaries, and Hg-contaminated areas in the Sacramento watershed (e.g., Clear Lake, Cache Creek, Davis Creek Reservoir). A range in sediment characteristics is sought, as well as a range in Hg and Se concentration and speciation. The species of Hg and Se within these sediments will be quantified

Table 1. Publications by the co-investigators pertaining to use of in vitro digestive fluid extraction to measure the bioavailability of selected contaminants.

POLYCYCLIC AROMATIC HYDROCARBONS

Weston, D. and L. Mayer. 1998. In vitro digestive fluid extraction as a measure of the bioavailability of sediment-associated polycyclic aromatic hydrocarbons: Sources of variation and implications for partitioning models. Environ. Toxicol. Chem. 17:820-829.

Weston, D. and L. Mayer. 1998. Comparison of in vitro digestive fluid extraction and traditional in vivo approaches as measures of polycyclic aromatic hydrocarbon bioavailability from sediments. Environ. Toxicol. Chem. 17:830-840.

MERCURY

Lawrence, A.L. and R.P. Mason. in review. The effects of organic matter on the bioavailability of organic and inorganic mercury to the estuarine amphipod Leptocheirus plumulosus. Environ. Sci. Technol.

Lawrence, A.L., K.M. McAloon, R.P. Mason, and L.M. Mayer. in press. Intestinal solubilization of particle-associated organic and inorganic mercury as a measure of bioavailability to benthic invertebrates. Environ. Sci. Technol.

OTHER TRACE METALS

Chen, Z. and L. Mayer. 1998. Mechanisms of Cu solubilization during deposit-feeding. Environ. Sci. Technol. 32:770-775.

Chen, Z., and L. Mayer. 1999. Sedimentary metal bioavailability determined by the digestive constraints of marine deposit feeders: gut retention time and dissolved amino acids, Mar. Ecol. Prog. Ser. 176:139-151.

Chen, Z. and L.M. Mayer. 1999. Assessment of sedimentary Cu availability: a comparison of biomimetic and AVS approaches. Environ. Sci. Technol.

ORGANIC AND INORGANIC CONTAMINANTS

Mayer, L., Z. Chen, R. Findlay, J. Fang, S. Sampson, L. Self, P. Jumars, C. QuetÈl, and O. Donard. 1996. Bioavailability of sedimentary contaminants subject to deposit-feeder digestion. Environ. Sci. Technol. 30:2641-2645.

Weston, D.W. 1999. Bioavailability of trace metals and organic compounds from Alameda Point sediments. Report to U.S. Navy from Univ. California, Berkeley, CA.

Weston, D.W. 1998. Measuring bioavailability of sediment-associated contaminants. Interagency Ecological Program for the Sacramento-San Joaquin Estuary Newsletter 11(3):12-13.

(e.g., inorganic and methyl-Hg, selenate, selenite, organic selenides, elemental Se). In evaluating these sediments with sturgeon digestive fluid, our primary intent is to test for consistent patterns (e.g., bioavailability of organic vs. inorganic forms).

We will do experiments to investigate how the bioavailability (i.e. degree of solubilization) of sediment-bound Hg and Se changes with organic matter content, AVS, Fe (oxide and sulfide phases), grain size, and the concentration of potentially "competing" metals (Cu, Cd, Zn), and the extent of interaction between these variables (e.g., the organic matter/AVS ratio; the AVS/Fe ratio). Our past work on solubilization of Hg from sediments has shown that matrix effects (e.g., organic carbon content, AVS) have an important impact on solubilization. Many field studies have shown that bioavailability of Hg is inversely proportional to sediment organic content (e.g., Langston, 1982; Muhaya et al., 1997). Initially we will look for correlations between digestive solubilization and sediment properties among the field-collected material, but eventually we will advance to a more manipulative approach in which we alter diet properties in order to observe the response in digestive solubilization. To obtain sediments of intermediate characteristics, natural sediments can be manipulated by the addition of muffled sand, organic matter, or by spiking with other metals.

We will establish if the extent of solubilization observed in vitro is equal to that observed in vivo, as found for PAH (Weston and Mayer, 1998). Demonstration of this equivalency with Hg and Se is important in establishing the environmental relevance of the digestive solubilization tasks. We will perform in vitro extraction using sturgeon digestive fluid, and also feed gelatin-encapsulated contaminated sediment to live sturgeon (the encapsulation technique has been used successfully in nutritional studies: S. Hung, UC Davis, pers. comm.). The fish will then be sacrificed, their digestive fluid extracted, and Hg and Se concentration in those fluids compared with concentrations achieved in vitro.

Task 2 - Digestive absorption: Bioaccumulation is a two-step process of digestive solubilization followed by absorption of the contaminant across the gut wall. Our solubilization technique incorporates only the first step, thus we must explicity test whether all the solubilized contaminant is subsequently absorbed. As the first step, we will measure the dietary absorption efficiency of Hg and Se (both organic and inorganic forms) by sturgeon. It is our expectation from the literature that absorption efficiencies for the organic forms will be quite high and considerably greater than those of inorganic Hg or Se (>50% - Phillips and Buhler, 1978; Lock, 1975; Luoma et al., 1992). Nevertheless, it is important to demonstrate these relative differences for our particular organisms as a foundation for other absorption tasks which follow.

Several sediments will be spiked with various forms of Hg and Se, and then encapsulated in gelatin. Prior to spiking the sediment, the matrix will be gamma irradiated to minimize the potential for any microbial transformation of the intended Hg or Se species. The spiked material will then be provided to the test organisms and absorption efficiency measured by whole body counting following a pulse chase procedure (Decho and Luoma, 1991). When measuring the amount of Hg absorbed, we will quantify Hg in the gut lining independently from the remainder of the fish tissue, as it has been shown that inorganic Hg accumulates in the intestinal lining whereas methyl-Hg rapidly passes across this barrier (Boudou et al., 1991). We will determine agreement between the proportion of Hg and Se solubilized in an in vitro extraction, and absorption efficiency by the whole animal. Close agreement would indicate that solubilization and not absorption is the factor limiting bioavailability, and suggests that solubilization alone would predict bioavailability.

To supplement the whole animal measures of absorption, we also plan gut perfusion experiments to allow more control over factors such as gut fluid composition or gut residence time than would be possible with the whole animals. We have performed short-term perfusion experiments to examine the transfer of inorganic Hg and MeHg across a gut membrane. The initial experiments have been performed with artificial seawater in the perfused gut, which is clearly not an adequate surrogate for the digestive fluid of fish. We will extend the gut perfusion experiments to these media and will use the gut of sturgeon in perfusion experiments.

We will also examine interactions between Hg and Se during solubilization and absorption. Our preliminary work has shown that the two elements seem to compete for the same digestive ligands, such that the extent of solubilization of one metal is dependent upon the concentration of

the other. By spiking sediments with both Hg and Se we will be able to quantify these interactions, which could prove to be quite important in the Delta where both Hg and Se can be present in sediments.

Task 3 - Synthetic cocktail development: Use of sturgeon digestive fluid is an essential step, but broad adoption of a digestive fluid approach to measuring bioavailabilty will require a broadly available surrogate. Task 3 will develop a synthetic cocktail based upon the enzymes and surfactants naturally present in digestive fluid and important to solubilization of Hg and Se. To understand solubilization mechanisms, we must first characterize gut fluid biochemistry. We will measure pH, low- and high-molecular weight amino acids (TCA precipitation followed by HPLC), selected enzymes (e.g. proteases), total dissolved organic carbon (by a Shimadzu 5000), dissolved lipids at class level (by an Iatroscan MK-5), and surfactancy using contact angles (a measure of potential for solubilization of hydrophobic substances; Mayer et al., 1997).

Our experience with cationic transition metals indicates that dissolved amino acids in gut fluids act as potent ligands to solubilize these metals (Chen and Mayer, 1998). In the case of Hg and Se we suspect that affinity for sulfur ligands will lead to cysteine and/or methionine as important to solubilization. Acidic conditions in the fish intestinal tract may play a role, and in fact multiple solubilization mechanisms may be involved, particularly for Se. We emphasize that this ligand identification has important implications for prediction of susceptible animal species. For example, methionine is depleted in detritus (Mayer et al., 1995), so that animals feeding on detritus may be less susceptible to inorganic Hg solubilization than animals feeding on cellular material (e.g., carnivores, who feed upon methionine-rich proteins). We will test for these ligands by performing blocking experiments on suspected binding sites, such as by adding an organic moiety that blocks the ligand site (e.g., sulfhydryl groups), and perform sediment incubations using both treated and untreated gut fluids.

The results from the solubilization mechanism work will enable us to design synthetic cocktails that contain relevant solubilizing agents. These agents might take the form of enzymes, inert proteins, or other biochemicals found to play a role in gut dissolution of Hg and Se. For example, we have found that cow blood protein, available commercially as bovine serum albumin, closely mimics digestive fluid in copper solubilization. We will validate the synthetic digestive fluid with side-by-side solubilization comparisons with actual sturgeon digestive fluid. Because of extensive work we have already done in this area using invertebrate digestive fluids, we will be able to establish which taxa beyond sturgeon the cocktail would adequately mimic.

Task 4 - Field demonstration: Mercury: To demonstrate the many applications of the digestive fluid approach in ecosystem restoration projects, two field tests of the technique are planned: one with Hg and one with Se. CALFED is currently supporting work by UC Davis to measure methyl-Hg production from reflooded wetlands, one component of which involves measuring Hg in resident fishes and invertebrates (e.g., goby, crayfish). Hg in their tissues should provide an integrated measure of exposure in each wetland and a measure of its bioavailability. We will collect sediment from these same sites and extract them with sturgeon digestive fluid and the synthetic surrogate. The UC Davis team expects bioavailability of Hg from the various wetlands to be variable depending on factors such as the time since flooding, salinity, soil organic matter quantity and type, etc. This task will establish if digestive fluid extraction predicts the relative Hg bioavailability from the various wetlands as quantified by body burden of the resident organisms.

Task 5 - Field demonstration: Selenium: CALFED is currently supporting a pond system by UC Berkeley investigators to remove Se from agricultural drainwater. The Se removed accumulates as sludges in anaerobic ponds, primarily as organoSe and elemental Se. With continued operation or if this technology is adopted elsewhere, there will be a need to dispose of these Se-rich sludges. Digestive fluid extraction technique provides a means to assess the bioavailability of Se from this material in order to guide sludge management decisions. Of particular interest is how transfer of the sludges from the current anaerobic system to an aerobic environment might affect bioavailability. While this could not be addressed with conventional biological measures of

bioavailability because of the impossibility of maintaining animals under anaerobic conditions, we will be able to determine how much of the Se is solubilized by digestive fluid and the surrogate synthetic cocktail when the extraction is performed anaerobically (under nitrogen). Repeating the extraction after oxygen exposure will establish if bioavailability is enhanced or mitigated by oxidation, and guide decisions on how the Se-rich sludges are to be disposed of.

Location of project - Work will be conducted in the laboratories of all project participants, but Tasks 1 includes field collection of sediment throughout the Delta and Sacramento and San Joaquin River watersheds. Task 4 will be done in the Delta. Task 5 will be done in selenium removal ponds near Firebaugh on the San Joaquin River.

ECOLOGICAL/BIOLOGICAL BENEFITS

Ecological/Biological Objectives

Ecosystem restoration efforts often change the rates of contaminant input into an environment or modify the physical and chemical conditions so as to make existing contaminants in that system more or less bioavailable. Flooding of agricultural lands to create wetland habitat can remobilize soil-bound contaminants. Changes in the input of organic matter, as might accompany water flow or land use changes through ecosystem restoration, would alter the bioavailability of contaminants capable of chemically interacting with organic carbon. Shifts to species assemblage utilizing different food sources may result in a contaminant being more or less bioavailable to the new species assemblage than it was to the prior assemblage. A thorough evaluation of the success of restoration efforts will often involve a need to assess the effect of those efforts on contaminant bioavailability.

Mercury is a major contaminant of concern in the Bay-Delta system because of on-going releases from Hg mines within the watershed and the historical discharge of elemental Hg from placer mining activities in the late 1800's. Fish advisories have been issued in San Francisco Bay because of high Hg in the tissue of recreationally exploited species. Selenium is also of major concern in the system due to inputs from agricultural irrigation in the San Joaquin drainage and oil refining in the North Bay. Selenium levels in biota of North San Francisco Bay have reached levels at which reproductive impairment is of serious concern, and sturgeon have specifically been identified as being at considerable risk (CALFED 1998). Given the high priority of these contaminants to resource management agencies, their tendency to be strongly particle-associated, and their propensity to bioaccumulate primarily through dietary routes, Hg and Se are ideal candidate toxicants for development and application of the digestive fluid approach. The technique, however, is not necessarily limited to these substances. As noted above we have already demonstrated its utility to PAH and certain metals. PCBs, organochlorine insecticides, and the more hydrophobic organophosphate pesticides all are likely to be suitable for use of the technique in quantifying bioavailability.

The digestive fluid extraction approach to quantifying bioavailability is of particular interest with regard to Se bioavailability because of the complex and poorly understood environmental chemistry of the element. Organic forms of Se are often considered the most bioavailable, but there is no accepted means to quantify their concentration in environmental samples. In the current CALFED-funded study by USGS on Se cycling in the Bay-Delta, organic Se is measured only by difference (total Se minus inorganic Se). Using digestive fluid extraction to measure bioavailability avoids the need to quantify each Se species, determine their relative bioavailabilities, and how this bioavailability may be influenced by physical/chemical conditions in the sediment. In digestive fluid extraction one need only quantify the total amount of Se in the digestive fluid, and an understanding of the complex underlying Se chemistry is not essential in predicting the extent that biota will accumulate Se from the sediment.

We focus on bioaccumulation of Hg and Se by demersal fish, with sturgeon as our model animal for several reasons. First, sturgeon are bottom-feeders foraging on deposit-feeding benthic invertebrates (e.g., polychaetes, molluscs, crustaceans), thus there is a clear trophic link between the fish and sedimentary Hg and Se. Sturgeon feed by vacuuming up the substrate, thus they

ingest significant quantities of sediment while foraging. 25-75% of the dry weight in the guts of sturgeon consists of sand and organic debris (Johnson et al., 1997). Second, sturgeon are harvested by recreational fishing so there is a clear connection to human Hg and Se exposure. They are specifically identified under the Proposal Solicitation Package Goal 3 as recreationally exploited species because of population declines and evidence of contamination. Sturgeon in the Bay contain 0.1-0.6 ppm Hg, and the vast majority of individuals exceed the State's threshold of concern of 0.14 ppm Hg (Ca. Dept. Fish and Game, unpub. data; SFBRWQCB, 1995; Davis et al., 1999). Third, there are readily available supplies of commercially cultured sturgeon, allowing us to obtain experimental animals at all life stages, in greater number, with greater ease, and with fewer seasonal limitations than would limit access to wild fish. We have worked with Stolt SeaFarms, a sturgeon producer near Galt, California, on other unrelated projects, and abundant supplies of digestive fluid can be obtained during routine processing of the cultured fish. It will not be necessary to collect from the limited supplies of wild fish in the Bay-Delta.

While our experiments will utilize digestive fluid of the white sturgeon, we do not view our results as limited in applicability to this species. Extrapolation to the green sturgeon is obvious, but in fact we have found that a wide variety of taxa have very similar digestive chemistry in the context of contaminant solubilization. In work with invertebrates we have found that with the exception of echinoderms, the digestive fluid of many diverse phyla have similar potential to solubilize zinc (Weston and Mayer, unpub. data). Thus, we believe our results will be broadly applicable to bottom-feeding fish and many invertebrates in general, and we will design the synthetic cocktail in this context. With an extensive data base we have developed on gut fluid compositon across many species, we should be able to reasonably predict which species for which a given cocktail is likely to mimic digestive chemistry.

Our ultimate objective is to provide CALFED and other resource management agencies a tool to quickly and easily measure contaminant bioavailability by a consistent method across a broad range of habitats. Our program has been designed in an ecosystem restoration context, and there are many applications of the technique in this context, but potential applications extend far beyond. The technique can be used for dredged material management, risk assessment, evaluating the success of remediation, and in fact, many contexts in which bioavailability of sediment-associated contaminants is of critical importance.

Linkages

There are two CALFED projects to which we have explicitly linked our study. The first is a UC Davis study (Suchanek and Slotten) of Hg bioavailability from restored wetlands. They are measuring bioavailability based on Hg body burden of resident fish and invertebrates. Our Task 4 will involve collection of sediment from their study sites, quantification of Hg bioavailability by digestive fluid extraction, and comparison of the two alternative bioavailability measures. A letter of support from Drs. Slotton and Suchanek is provided at the end of this proposal.

The second CALFED project which we have integrated into our work is the ABSR selenium removal system being developed by UC Berkeley investigators (Oswald) near Panoche, California. The ABSR ponds will, over time, accumulate a Se-rich sludge. In its current location at the bottom of an anaerobic pond, it poses little threat to biota, but ultimately disposal alternatives will have to be considered. Our study will evaluate the bioavailability of Se in this sludge, and specifically how transition from an anaerobic to aerobic environment affects this bioavailability. A letter of support from Dr. Oswald is provided at the end of this proposal.

Our study will also share areas of common research interests with the CALFED-funded study on Se impacts in the Bay-Delta ecosystem (Luoma et al.). This study will measure Se bioaccumulation by primary consumers, several invertebrate species and fish. Our studies on absorption of various Se forms by sturgeon, and the constraints imposed by the initial digestive solubilization, should be of interest to these investigators. In addition, as a component of their project, Dr. Hinton of UC Davis will be studying the effect of Se on sturgeon reproduction and development. His interests in toxicological effects is a logical extension of our interests in the bioaccumulation process.

Finally, CALFED will soon be initiating a study of Hg impacts in the Bay-Delta watershed (Stephenson et al.). This study involves measuring Hg bioavailability, but does so by using "methyl-Hg/total Hg ratios in sediment to give a first order estimation of methylation potential which is a good proxy for bioavailability" (Stephenson et al., Hg proposal to CALFED, p. 7). While we agree that methyl-Hg is more bioavailable than inorganic Hg, we have extensive data that shows more than half the methyl-Hg in sediment is not bioavailable, the bioavailable fraction of even methyl-Hg is highly variable among sediments, and methylHg concentrations in sediments do not correlate well with body burden of resident organisms. Thus we believe that our more direct measures of Hg bioavailability (both digestive solubilization and the sturgeon exposures done in support of it) can provide insights into the sediment conditions which enhance or mitigate methyl-Hg bioavailability, and will prove invaluable in helping the other investigators interpret their observations. Drs. Suchanek and Slotton are principal investigators on this project as well, and the linkages between our studies are discussed in their letter of support.

Our laboratory studies of contaminant solubilization and absorption will be done with white sturgeon, and results would certainly be directly relevant to green sturgeon. The work is most relevant to the contaminant strategic objectives and targets (p. 506, 2/99 ERP), and strategic objectives and targets for bottom feeding fishes such as green sturgeon (p. 205), white sturgeon (p. 402), and splittail (p. 209). We note, however, that it is difficult to identify the species-specific Ecosystem Restoration Program Goals which the project addresses since the work crosses species boundaries. We believe the proposed study will provide considerable insight into food chain transport of Hg and Se in general, and can be used as a risk assessment tool that benefits all aquatic species.

System-Wide Ecosystem Benefits

We have limited the proposed work to an evaluation of Hg and Se bioavailability, but there is no fundamental reason why the technique could not be applied to other sediment-associated contaminants. Pesticides are an obvious example. Chlorpyrifos has recently been found in sediments of urban storm drains and on suspended particulate matter in the Delta. We are currently planning work with USGS to examine the bioavailability of chlorpyrifos from these suspended sediments. In addition Central Valley soils contain organochlorine residues from application of these pesticides decades ago, and the digestive fluid approach could be useful in determining the extent of bioavailability of these residues after all these years. The digestive fluid approach would be useful in assessing bioavailability of virtually any sediment-associated toxicant, including heavy metals, hydrocarbons and PCBs.

We have focused the proposed work on topic areas of specific interest to CALFED, but development of the digestive fluid technique will provide a tool of value to scientists and many resource management agencies. Both state agencies (e.g., State Water Resources Control Board, Dept. of Fish and Game) and federal agencies (e.g., Environmental Protection Agency, Army Corps of Engineers) have many on-going investigations or monitoring programs in which there is a need to assess the risk of contaminated sediments. The tools provided by the proposed work would be valuable to these agencies, and provide data that would be far more ecologically realistic and defensible than those based on conventional chemical analysis of contaminated sediments alone.

Compatibility with non-ecosystem objectives

The need to measure contaminant bioavailability and the approach we propose to do so is applicable to several CALFED Bay-Delta Program objectives. The Ecosystem Quality objective is to improve habitats to support diverse and valuable plant and animal species. Many potential habitats contain residues of anthropogenic substances that are potentially toxic and could prevent the establishment of sustainable populations of desired species (e.g. pesticide residues in soils, Hg in sediments from hydraulic mining activities). However, the mere presence of these contaminants does not in itself indicate that the habitat is unsuitable and the bioavailability of these substances is an equally important issue that must be addressed in order to determine if populations are sustainable in the improved habitat. The Water Quality objective will also benefit from the

proposed work. Not only will the information be valuable in protecting water quality for resident aquatic species, but there are clear implications to recreational fishing and human health. There are currently fishing advisories in effect in San Francisco Bay because of Hg contamination, and the proposed work will provide data on how these fish accumulate Hg from contaminated sediments.

TECHNICAL FEASIBILITY AND TIMING

Environmental management decisions often are based on conventional chemical analyses of contaminant concentrations in sediments. Conventional chemical measures, however, are generally useless in predicting contaminant bioavailability. Conventional extraction techniques, typically using a strong acid for contaminants such as Hg and Se, are intended to extract all of the contaminant, and therefore far overestimate the bioavailable fraction. For example, our preliminary work has shown that <10% of inorganic mercury and <20% selenite are extractable from sediment by digestive fluid, indicating that conventional extraction techniques overestimate bioavailability by at least a factor of 5.

Even if chemical analyses are used to quantify a specific species that is more bioavailable, such as methyl-Hg, these data are generally inadequate bases on which to base management decisions. We have several data sets that indicate methyl-Hg concentrations are not reliable predictors of the potential for Hg bioaccumulation. Methyl-Hg concentrations in sediments are uncorrelated to Hg body burden of killifish and many benthic invertebrate species in our studies in Chesapeake Bay and Texas (Mason, unpub. data). In digestive fluid extractions of sediment we have found the the proportion of methyl-Hg that is bioavailable may vary from 2-38% depending on sediment organic carbon, AVS, and other factors. In fact, we have found that the proportion of methyl-Hg solubilized in the fluid is an excellent predictor of the ability of benthic amphipods to accumulate the methyl-Hg from the sediment (Lawrence and Mason, in review).

The other alternative to chemical analyses to predict contaminant bioavailability is a biological measure such as bioaccumulation testing using either resident or laboratory-exposed organisms. While the resultant data are useful in ecosystem restoration decisions, the use of animal-based measures presents some significant difficulties. Often it is necessary to measure bioavailability over a broad environmental gradient that exceeds the tolerance range of any one species,thus it is impossible to obtain a consistent bioavailability measure across the range of conditions (e.g., the RMP bioaccumulation monitoring program has to use one bivalve species in San Francisco Bay and a second species in the Delta). In addition, it may be desirable to measure bioavailability under conditions where animal exposure is impossible, such as Task 5 proposed above in which we will establish how Se bioavailability is dependent upon the anaerobic conditions under which the sludges are maintained. Finally, laboratory bioaccumulation testing is among the most expensive ways to evaluate contaminated sediments because of both the extensive chemical analytical needs and the long exposure period that is required.

The digestive fluid extraction approach has as much biological relevance as biological measures of bioavailability, but with the speed and simplicity of a chemical extraction. Moreover, it provides results consistent with the traditional biological approaches to quantifying bioavailability (Weston and Mayer, 1998; Weston, 1999; Lawrence and Mason, in review).

MONITORING AND DATA COLLECTION METHODOLOGY

Our monitoring and data collection information is summarized in Table 2, and many of the details have already been provided earlier in this proposal. Table 2 is divided into five sections based on biological/ecological objectives which correspond to the five tasks described in the Project Description section:

- Quantify digestive solubilization of Hg and Se
- Quantify digestive absorption of Hg and Se
- Synthetic cocktail development
- Field demonstration: mercury
- Field demonstration: selenium

Table 2. Monitoring and Data Collection Information

Hypothesis/Question Evaluated	Monitoring Parameters and Data Collec. Approach	Data Evaluation Approach	Comments/ Data priority
Biological/Ecological Objective: Quantify digestive solubilization of Hg and Se			
How much Hg/Se in natural sediments would be available to an organism ingesting that sediment?	Extensive field collections of sediments in Delta, Rivers and tributaries; extract by sturgeon digestive fluid.	Determine proportion of total Hg/Se that is extractable.	High
How is digestive solubilization dependent on chemical species?	Extensive field collections of sediments in Delta, Rivers and tributaries; extract by sturgeon digestive fluid.	Quantify Hg/Se in sediments according to chemical form and determine extracability for each.	High
On which environmental factors is Hg/Se bioavailability dependent?	As above. Also laboratory manipulations of sediment properties to observe effects on solubilization.	Correlations between sediment properties and solubilization.	High
Is in vitro solubilization equal to in vivo solubilization?	Parallel experiments in which sediment is extracted in vitro and also fed to sturgeon which are then sacrificed to analyze digestive fluid.	Agreement between in vitro and in vivo approach in amount of Hg/Se solubilization.	High
Biological/Ecological Objective: Quantify digestive absorption of Hg and Se			
How efficiently are various chemical forms of Hg/Se absorbed in the gut.	Feed sturgeon Hg/Se labelled sediments and measure absorption by pulse chase. Also measure absorption by perfused guts.	Determine proportion of each Hg/Se form absorbed.	High
Is the proportion of Hg/Se solubilized equal to that absorbed in the gut?	Feed sturgeon Hg/Se labelled sediments and measure absorption by pulse chase. Also measure absorption by perfused guts.	Compare proportion of Hg/Se solubilized to the proportion absorbed.	High
Do Hg and Se interact competitively or in other ways during solubilization and absorption?	Measure solubilization and absorption of Hg and Se independently and when in the same sediment concurrently.	Compare solubilization and absorption efficiencies for statistical differences.	High

Table 2. Monitoring and Data Collection Information (continued)

Hypothesis/Question Evaluated	Monitoring Parameters and Data Collection Approach	Data Evaluation Approach	Comments/ Data priority
Biological/Ecological Objective: Synthetic cocktail development			
What agents in digestive fluid are responsible for Hg/Se solubilization?	Analysis of enzymes, surfactancy, pH, DOC, lipids, etc. in digestive fluid. Blocking of suspected ligands and test for effect on solubilization.	Chemical characterization of gut fluid. Statistical comparison of solubilization with and without blocking agent.	High
Can a synthetic cocktail be formulated to mimic Hg/Se solubilization.	Use characterization results to develop a synthetic version using commercially available enzymes, etc.	Compare solubilization rates and extents over multiple sediments and Hg/Se species to evaluate success of biomimetic solution.	High
Biological/Ecological Objective: Field demonstration: mercury			
How does digestive fluid extraction compare to other measures of Hg bioavailability when used in an ecosystem restoration context?	Collect sediment from multiple restored wetlands, and measure Hg bioavailability from these sediments using actual and synthetic digestive fluids.	Compare solubilization estimates of bioavailability at each wetland to bioaccumulation by resident species. Compare results from the two extractants.	High
Biological/Ecological Objective: Field demonstration: selenium			
Can digestive fluid extraction be used to assess Se bioavailability from sludges generated by a Se removal technology?	Collect sludges from Se removal ponds and measure bioavailability both anaerobically and aerobically using actual and synthetic digestive fluids.	Compare solubilization under the both redox conditons. Compare results from the two extractants.	High

Our primary data collection method will be in vivo digestive fluid extraction. The amount of contaminant desorbed from the sediment of interest and solubilized in the digestive fluid or synthetic surrogate is the critical parameter by which we measure bioavailability. We wish to emphasize that while the approach is novel in that the first paper was published only in 1996 (Mayer et al., 1996), its scientific validity has been established by the numerous papers that we have published in the peer-reviewed literature since that time (as listed previously in Table 1). In addition the Environmental Protection Agency, Office of Naval Research, Department of Defense, US Navy, UC Toxic Substances Research and Teaching Program, and the UC Water Resources Center have all provided funding to us at various times to develop the approach, thus showing further demonstration of its recognized potential.

Our analytical needs include gamma counting (when using radioactive Hg and Se in laboratory experiments), gold-amalgamation/cold vapor atomic fluorescence (CVAFS) for Hg analyses, and PSA hydride generation atomic fluorescence for Se. Dr. Mason will be responsible for performing these analyses using the facilities at the Chesapeake Biological Laboratory's Trace Metal Analytical Facility of the University of Maryland. This laboratory has extensive experience in both Hg and Se measurements from environmental samples. In particular, its Hg capability is highly regarded and some of its procedures have formed the basis for EPA Method 1631 for Hg analyses.

Some absorptive experiments that require greater manipulation of certain variables will be done with perfused gut segments. The use of perfused tissues to study absorptive processes is well established, and we have used it to study Hg uptake across the blue crabs. Quantification of absorption efficiency in whole sturgeon (juveniles) will be done by pulse chase methods. These procedures are well-established, and specifically most widely used for trace metals (Decho and Luoma, 1991; and many others). The advantage of using radioactive Hg or Se for this purpose is that Hg and Se body burden in the sturgeon can be measured non-destructively by gamma counting. The radioactivity in an individual can be measured after ingesting the contaminated material, that individual is returned to the aquarium to allow it to clear its gut, and then the same individual can be counted for radioactivity again. The difference between the two counts can be used to derive absorption efficiency.

LOCAL INVOLVEMENT

As discussed above and as indicated by the letters of support we have integrated our efforts with those of investigators working throughout the Bay-Delta, and particularly those involved in related CALFED projects. The type of work proposed does not lend itself to involvement by local government agencies. State resource management agencies would have considerable interest in the results of the work, and staff of these agencies will be reached by presentations in CALFED-sponsored forums and local environmental conferences (e.g. San Francisco Bay State of the Estuary Conference, NorCal chapter of the Society for Environmental Toxicology and Chemistry). In addition we will publish the results in both the peer-reviewed literature, and also as a chapter in the annual Regional Monitoring Program report of the San Francisco Estuary Institute. We have also published in the past on the digestive fluid method in the IEP Newsletter (Vol. 11, No. 3, 1998), and would anticipate doing so in the proposed project as well.

A local business, Stolt SeaFarms, will provide the juvenile sturgeon to be used in this study. We have worked with Stolt for several years on a project unrelated to that proposed, and they welcome the opportunity to use their cultured fish in a project that will benefit both the wild stock and the Bay-Delta ecosystem as a whole.

The majority of the work proposed will be conducted in university laboratories, and thus notification of affected counties is not relevant to these tasks. There are two field demonstration tasks, the first of which (mercury) will be done at sites throughout the Delta in Solano and San Joaquin counties; and the second of which (selenium) will be done in ponds near Firebaugh, Madera County. As requested by CALFED, the County Board of Supervisors and Planning Department of these counties have been notified, and a copy of the letter is attached.

COST

Budget

The budget for the proposed work is shown in the attached tables with each page representing the budget for a single institution. Univ. Maryland and Univ. Maine are participating in this project as subcontractors to the University of California, and the total dollar value of their subcontracts are included in the Univ. California budget under the "other direct cost" category.

Salary costs include those for the principal investigators, post-doctoral researchers (Maine and Maryland), graduate student (Maryland), technicians (Maryland and California), and undergraduate assistants (California). Fringe benefit rates vary depending on the type of position.

Materials and acquisitions largely consists of expendable supplies, the largest fraction being those associated with chemical analyses of Hg and Se, and the purchase of radioactive Hg and Se. The only equipment item included in the budget is the purchase of a gamma counter at UC Berkeley (approx. $35,000). The gamma counting instrumentation currently available is inadequate to measure whole juvenile fish and can not simultaneously distinguish activity of Hg and Se, both of which are needs essential to this project.

Indirect costs vary among the institutions. Univ. Maryland indirect costs are 43% of total direct costs. Univ. Maine indirect costs are 45% of total direct costs. Univ. California indirect costs depend upon whether the source of funds for this project are from the state or federal government. If state funds are provided, indirect costs are 10% of direct costs excluding equipment, and the 10% charge is applied to the full value of all subcontracts. If federal funds are provided, indirects costs are 50.4% of direct costs excluding equipment, and only the first $25,000 of each subcontract is subject to the 50.4% indirect cost. In preparing the budgets, indirect costs on the first $25,000 of each subcontract were placed in the first quarters and in the first tasks each subcontractor participates in until the $25,000 limit was reached (i.e., UCB indirect costs on the Maryland subcontract in Task 1, quarters 1 and 2; Maine subcontract in Task 3, quarters 1 and 2).

The Project Management Task, budgeted only for the prime contractor (UCB), includes time required to prepare quarterly reports, respond to requests for information from CALFED or member agencies, give oral presentations to CALFED, track progress by the subcontractors, and includes costs to travel to one of the subcontractor's institutions each year to coordinate research efforts.

Schedule

The project will extend over three years, and for purposes of scheduling and budgeting is assumed to start Oct. 1, 1999 and be completed by Sept. 30, 2002. The schedule for each task is shown below:

	1999][2000][2001][2002
	ONDJFMAMJJASONDJFMAMJJASONDJFMAMJJAS
Task 1- Digestive solubilization	xxxxxxxxxxxxxxxxxx
Task 2 - Digestive absorption	xxxxxxxxxxxxxxxxxxxxxx
Task 3 - Cocktail development	xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx
Task 4 - Field demonstration: Hg	xxx
Task 5 - Field demonstration: Se	xxx

The project will initially concentrate on the digestive solubilization work and then later shift to an emphasis on absorptive constraints on bioaccumulation. Work to establish the mechanisms of Hg and Se solubilization by digestive fluid and then to develop a synthetic cocktail based upon the relevant mechanisms is anticipated to be a lengthy and iterative process that will rely upon information gathered in the other tasks throughout the project. Field demonstration tasks are intended to occur at the end of the project since they rely upon the cocktail produced in Task 3. Manuscripts for publication in the peer-reviewed literature and CALFED technical documents will be prepared throughout the project, generally near the end of the period allotted for each task

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COST SHARING

Dr. Mayer has a 9 month academic appointment, and will provide one month each year of his state-supported (Maine) time to the CALFED project. Salary costs, fringe benefits, and associated indirect costs for this in-kind cost sharing have a value of $47,503.

Dr. Weston has received funding from the University of California Water Resources Center to further develop the digestive fluid approach as an environmental management tool, and much of the work conducted through that grant will be directly applicable to the CALFED project. Work will be conducted from July 1999 through June 2001. The value of this award is $53,000, and is offered as in-kind cost sharing, supported by the state of California.

The total value of all cost-sharing on the proposed project is $100,503.

APPLICANT QUALIFICATIONS

The proposed work represents a collaboration among investigators at the University of California, Berkeley, the University of Maine, and the University of Maryland. Each participant brings unique talents to the project and they were included in this project because of extensive directly relevant research experience. The in vitro solubilization concept was originally proposed in 1996 by Dr. Mayer, and his lab has done a great deal of work on trace metal and PAH bioavailability using the technique. Dr. Weston has extensively applied the technique to polycyclic aromatic hydrocarbons, and has been validating the procedure with whole animal measures of absorption and bioaccumulation. Dr. Mason's expertise lies in Hg bioavailability, and he has used the technique as a tool to quantify this bioavailability. Drs. Mayer and Weston have collaborated on other projects, as have Drs. Mason and Mayer, and we anticipate the proposed collaboration will be as productive as those already completed.

Dr. Donald Weston will act as Project Manager for the proposed work, and will be responsible for primary contact with CALFED and the coordination of tasks among the other project participants. He also will have specific technical responsibilities in Task 1 and 2, and primary responsibility for the field demonstrations of Tasks 4 and 5. Dr. Weston is an ecotoxicologist with nearly 20 years experience. His research has focused on issues pertaining to bioaccumulation of sediment-associated contaminants, and has included work on bioavailability, trophic transfer of contaminants, sediment toxicity testing, and toxicant effects on benthic community structure. He has done a great deal of work in recent years on the digestive fluid approach to measuring bioavailability including comparison of the technique to more traditional techniques, and an interphyletic study in which the digestive fluids of 20 species were compared in their ability to solubilize both a polycyclic aromatic hydrocarbon and trace metal. His work on digestive fluid has been funded by the US Environmental Protection Agency, US Navy, UC Toxic Substances Research and Teaching Program, and the UC Water Resources Center. Collaborations with Lawrence Berkeley National Laboratories, US Geological Survey, and US Army Corps of Engineers are in the initial planning stages. His research group consists of a technician and two graduate students, one of whom is studying Hg toxicity and the other of whom is conducting research on Se bioavailability.

Dr. Robert Mason will have responsibility for specific components of Tasks 1, 2 and 3, and in additional will provide Hg and Se analytical assistance to all project participants. He has been involved in innovative mercury research for the past 12 years, obtaining his Ph.D. degree under the mentorship of Dr. William Fitzgerald, an internationally known Hg researcher. He has been involved in the development of the recent analytical and methodological techniques that now allow the uncompromised determination of mercury and methylmercury concentrations in natural waters, sediments and biota. He has a total of 40 Hg-related publications in the last 10 years, including a paper in press that is directly relevant to the topic of this proposal (Lawrence, McAloon, Mason and Mayer: Intestinal solubilization of particle-associated organic and inorganic mercury as a measure of bioavailability to benthic organisms; Environmental Science and Technology, in press). Mason's research focuses on Hg biogeochemistry and toxicology, but also

includes work on the metalloids, particularly arsenic and selenium, and the more toxic trace metals. Mason's research has recently focused on understanding the factors controlling Hg and methyl-Hg bioaccumulation into aquatic organisms, both in the benthic and pelagic realm, through research funded by the EPA, NOAA, and the Maryland DNR (2 recent MS Theses; 4 papers published or in press, one in review). Mason's group is involved in an additional project looking into the factors controlling Hg, methyl-Hg, arsenic, selenium and cadmium accumulation into freshwater invertebrates and fish, and a paper has recently been submitted to Archives of Environmental Contamination and Toxicology. Mason has a current collaboration through the University of Santa Cruz/San Francisco Estuary Institute to measure Hg and methyl-Hg in waters of San Francisco Bay. His group currently consists of 4 students, a technician and two post-doctoral researchers, and has all the necessary expertise for the studies under the current proposal

Dr. Lawrence Mayer will have primary responsibility for investigation of the mechanisms of Hg and Se digestive solubilization and the development of synthetic cocktails to mimic the natural processes (Task 3). He is a Professor of Oceanography at the University of Maine, specializing in marine biogeochemistry. He has performed extensive research into the bioavailability of materials at the land-sea interface, including nutritional and toxic substances. The toxics work has included both metals and organics. Coupled to the biogeochemical work has been characterization of organismal physiology as it relates to bioavailability - for example, characterizing the types of enzyme or surfactants that are responsible for solubilization of various nutritional or toxic substances. Projects funding this work have been obtained from the U.S. Department of Defense, Environmental Protection Agency, National Science Foundation, and the Maine Center for Innovation in Biotechnology. In the 23 years since his Ph.D., he has published 75 papers in a variety of chemical, biological and geological journals.

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Principal Investigator: **Donald P. Weston, Ph.D.**
University of California, Berkeley

QUARTERLY BUDGET
University of California, Berkeley

If funded with Federal funds:

Tasks	Quarter 1 Oct-Dec 1999	Quarter 2 Jan-Mar 2000	Quarter 3 Apr-Jun 2000	Quarter 4 Jul-Sept 2000	Year 1 Total
Task 1	43,830	40,489	76,597	38,732	199,648
Task 2	-	-	-	-	-
Task 3	20,506	19,361	7,167	13,633	60,667
Task 4	-	-	-	-	-
Task 5	-	-	-	-	-
Project Mgt. Task	4,103	2,599	2,599	2,599	11,900
Grand Totals	**68,439**	**$ 62,449**	**$ 86,363**	**$ 54,964**	**$ 272,215**

Tasks	Quarter 5 Oct-Dec 2000	Quarter 6 Jan-Mar 2001	Quarter 7 Apr-Jun 2001	Quarter 8 Jul-Sept 2001	Year 2 Total
Task 1	35,609	-	-	-	35,609
Task 2	3,785	43,474	46,792	39,141	133,192
Task 3	14,134	14,134	14,134	14,134	56,536
Task 4	-	-	-	-	-
Task 5	-	-	-	-	-
Project Mgt. Task	4,153	2,649	2,649	2,649	12,100
Grand Totals	**57,681**	**$ 60,257**	**$ 63,575**	**$ 55,924**	**$ 237,437**

Tasks	Quarter 9 Oct-Dec 2001	Quarter 10 Jan-Mar 2002	Quarter 11 Apr-Jun 2002	Quarter 12 Jul-Sept 2002	Year 3 Total	Grand Totals
Task 1	-	-	-	-	-	235,257
Task 2	26,480	3,501	-	-	29,981	163,173
Task 3	32,903	32,902	14,451	14,451	94,707	211,910
Task 4	-	-	32,719	-	32,719	32,719
Task 5	-	-	-	32,418	32,418	32,418
Project Mgt. Task	4,202	2,698	2,698	2,698	12,296	36,296
Grand Totals	**63,585**	**$ 39,101**	**$ 49,868**	**$ 49,567**	**$ 202,121**	**$ 711,773**

Principal Investigator: **Donald P. Weston, Ph.D**
University of California, Berkeley

QUARTERLY BUDGET
University of California, Berkeley

If funded with State funds:

Tasks	Quarter 1 Oct-Dec 1999	Quarter 2 Jan-Mar 2000	Quarter 3 Apr-Jun 2000	Quarter 4 Jul-Sept 2000	Year 1 Total
Task 1	32,056	33,218	72,125	34,260	171,659
Task 2	-	-	-	-	-
Task 3	14,997	14,770	7,167	13,633	50,567
Task 4	-	-	-	-	-
Task 5	-	-	-	-	-
Project Mgt. Task	3,001	1,901	1,901	1,901	8,704
Grand Totals	**$ 50,054**	**$ 49,889**	**$ 81,193**	**$ 49,794**	**$ 230,930**

Tasks	Quarter 5 Oct-Dec 2000	Quarter 6 Jan-Mar 2001	Quarter 7 Apr-Jun 2001	Quarter 8 Jul-Sept 2001	Year 2 Total
Task 1	30,583	-	-	-	30,583
Task 2	3,785	38,943	42,261	34,610	119,599
Task 3	14,134	14,134	14,134	14,134	56,536
Task 4	-	-	-	-	-
Task 5	-	-	-	-	-
Project Mgt. Task	3,037	1,937	1,937	1,937	8,848
Grand Totals	**$ 51,539**	**$ 55,014**	**$ 58,332**	**$ 50,681**	**$ 215,566**

Tasks	Quarter 9 Oct-Dec 2001	Quarter 10 Jan-Mar 2002	Quarter 11 Apr-Jun 2002	Quarter 12 Jul-Sept 2002	Year 3 Total	Grand Totals
Task 1	-	-	-	-	-	202,242
Task 2	21,403	3,501	-	-	24,904	144,503
Task 3	32,903	32,902	14,451	14,451	94,707	201,810
Task 4	-	-	28,530	-	28,530	28,530
Task 5	-	-	-	28,310	28,310	28,310
Project Mgt. Task	3,073	1,973	1,973	1,973	8,992	26,544
Grand Totals	**$ 57,379**	**$ 38,376**	**$ 44,954**	**$ 44,734**	**$ 185,443**	**$ 631,939**

I-018962

Principal Investigator: Donald P. Weston, Ph.D.
University of California, Berkeley

Subcontracts:

TOTAL BUDGET
University of Maryland

Tasks	Direct Labor Hours	Direct Salary & Benefits	Service Contracts	Material & Acq. Costs	Misc. & Other Direct Costs	Total Direct Costs	Indirect Costs	Total Costs
Task 1	1,626	56,485	1,315	8,000	1,250	67,050	28,831	95,881
Task 2	1,620	52,715	1,284	10,000	1,500	65,499	28,165	93,664
Task 3	598	20,800	506	4,000	500	25,806	11,097	36,903
Task 4	311	9,490	235	2,000	250	11,975	5,149	17,124
Task 5	311	9,490	235	2,000	250	11,975	5,149	17,124
Grand Totals	**4,466**	**$ 148,980**	**$ 3,575**	**$ 26,000**	**$ 3,750**	**$ 182,305**	**$ 78,391**	**$ 260,696**

TOTAL BUDGET
University of Maine

Tasks	Direct Labor Hours	Direct Salary & Benefits	Service Contracts	Material & Acq. Costs	Misc. & Other Direct Costs	Total Direct Costs	Indirect Costs	Total Costs
Task 1	130	3,286	-	725	450	4,461	2,007	6,468
Task 2	-	-	-	-	-	-	-	-
Task 3	3,110	84,931	-	17,275	9,799	112,005	50,402	162,407
Task 4	-	-	-	-	-	-	-	-
Task 5	-	-	-	-	-	-	-	-
Grand Totals	**3,240**	**$ 88,217**	**$ -**	**$ 18,000**	**$ 10,249**	**$ 116,466**	**$ 52,409**	**$ 168,875**

QUARTERLY BUDGET
University of Maryland

Tasks	Quarter 1 Oct-Dec 1999	Quarter 2 Jan-Mar 2000	Quarter 3 Apr-Jun 2000	Quarter 4 Jul-Sept 2000	Year 1 Total
Task 1	18,072	20,348	18,480	22,083	78,983
Task 2	-	-	-	-	-
Task 3	-	-	-	-	-
Task 4	-	-	-	-	-
Task 5	-	-	-	-	-
Project Mgt. Task	-	-	-	-	-
Grand Totals	**$ 18,072**	**$ 20,348**	**$ 18,480**	**$ 22,083**	**$ 78,983**

Tasks	Quarter 5 Oct-Dec 2000	Quarter 6 Jan-Mar 2001	Quarter 7 Apr-Jun 2001	Quarter 8 Jul-Sept 2001	Year 2 Total
Task 1	16,898	-	-	-	16,898
Task 2	3,785	26,605	29,923	22,272	82,585
Task 3	-	-	-	-	-
Task 4	-	-	-	-	-
Task 5	-	-	-	-	-
Project Mgt. Task	-	-	-	-	-
Grand Totals	**$ 20,683**	**$ 26,605**	**$ 29,923**	**$ 22,272**	**$ 99,483**

Tasks	Quarter 9 Oct-Dec 2001	Quarter 10 Jan-Mar 2002	Quarter 11 Apr-Jun 2002	Quarter 12 Jul-Sept 2002	Year 3 Total	Grand Totals
Task 1	-	-	-	-	-	95,881
Task 2	7,578	3,501	-	-	11,079	93,664
Task 3	18,452	18,451	-	-	36,903	36,903
Task 4	-	-	17,124	-	17,124	17,124
Task 5	-	-	-	17,124	17,124	17,124
Project Mgt. Task	-	-	-	-	-	-
Grand Totals	**$ 26,030**	**$ 21,952**	**$ 17,124**	**$ 17,124**	**$ 82,230**	**$ 260,696**

I-018964

QUARTERLY BUDGET
University of Maine

Tasks	Quarter 1 Oct-Dec 1999	Quarter 2 Jan-Mar 2000	Quarter 3 Apr-Jun 2000	Quarter 4 Jul-Sept 2000	Year 1 Total
Task 1	-	-	6,468	-	6,468
Task 2	-	-	-	-	-
Task 3	13,634	13,633	7,167	13,633	48,067
Task 4	-	-	-	-	-
Task 5	-	-	-	-	-
Project Mgt. Task	-	-	-	-	-
Grand Totals	**$ 13,634**	**$ 13,633**	**$ 13,635**	**$ 13,633**	**$ 54,535**

Tasks	Quarter 5 Oct-Dec 2000	Quarter 6 Jan-Mar 2001	Quarter 7 Apr-Jun 2001	Quarter 8 Jul-Sept 2001	Year 2 Total
Task 1	-	-	-	-	-
Task 2	-	-	-	-	-
Task 3	14,134	14,134	14,134	14,134	56,536
Task 4	-	-	-	-	-
Task 5	-	-	-	-	-
Project Mgt. Task	-	-	-	-	-
Grand Totals	**$ 14,134**	**$ 14,134**	**$ 14,134**	**$ 14,134**	**$ 56,536**

Tasks	Quarter 9 Oct-Dec 2001	Quarter 10 Jan-Mar 2002	Quarter 11 Apr-Jun 2002	Quarter 12 Jul-Sept 2002	Year 3 Total	Grand Totals
Task 1	-	-	-	-	-	6,468
Task 2	-	-	-	-	-	-
Task 3	14,451	14,451	14,451	14,451	57,804	162,407
Task 4	-	-	-	-	-	-
Task 5	-	-	-	-	-	-
Project Mgt. Task	-	-	-	-	-	-
Grand Totals	**$ 14,451**	**$ 14,451**	**$ 14,451**	**$ 14,451**	**$ 57,804**	**$ 168,875**

I-018965

University of Maryland
Center for Environmental Science
Chesapeake Biological Laboratory
P.O. Box 38
Solomons, Maryland 20688-0038

Ref. No. [UMCES]CBL 99-0033

PROPOSAL

A Subcontract Submitted to the University of California-Berkeley

A New Approach to Assess the Effect of Ecosystem Restoration Efforts on Contaminant Bioavailability

April 6, 1999

Robert P. Mason, Principal Investigator
Chesapeake Biological Laboratory

APPROVALS:

K. R. Tenore /RSA

Kenneth R. Tenore, Director
Chesapeake Biological Laboratory

Donald F. Boesch

Donald F. Boesch, President
Center for Environmental Science

I-018966

PROPOSAL TO:	University of California Sponsored Programs Office 336 Sproul Hall Berkeley, CA 94720-5940
FROM:	Office of Research and Sponsored Programs Room 408 University of Maine 5717 Corbett Hall Orono, ME 04469-5717
SUBJECT:	Assess Effects of Ecosystem Restoration - Bioavailability
PERIOD:	10/01/1999 to 09/30/2002
FUNDS REQUESTED:	$168,875
PRINCIPAL INVESTIGATOR:	Lawrence Mayer University of Maine School of Marine Sciences Darling Marine Center Clarks Cove Road Walpole, ME 04573
APPROVED FOR SUBMISSION:	Paul D. Uttormark Paul D. Uttormark, Director Office of Research and Sponsored Programs 4-8-99 Date

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I-018967

UNIVERSITY OF CALIFORNIA, DAVIS

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DEPARTMENT OF ENVIRONMENTAL SCIENCE AND POLICY

ONE SHIELDS AVENUE
DAVIS, CALIFORNIA 95616-8576

4/13/99

Dear CALFED administrators and proposal reviewers,

We are writing in support of the following April 1999 proposal submission entitled: **A new approach to assess the effect of ecosystem restoration efforts on contaminant bioavailability** Principal Investigators Donald Weston, Robert Mason, and Lawrence Mayer.

We believe that this proposed work will provide a valuable addition to the CALFED effort in the area of Water Quality. We are impressed with the potential ecological relevance of this novel approach and can see a great potential benefit to existing CALFED Water Quality related projects. In particular, our extensive involvement in questions of heavy metal bioavailability in the Bay-Delta system and its watershed could link very strongly to this proposed work. In our ongoing CALFED project looking at the potential methyl mercury consequences of Bay-Delta wetlands restoration efforts, a major focus involves the determination of relative methyl mercury production and associated mercury bioavailability in a wide variety of Delta sites which define a range of restoration endpoints in the system. We are going to great lengths to utilize ecologically relevant measures. Collaborative work by Weston, Mason, and Mayer would provide an additional measure that could be highly relevant to these questions. These investigators have expressed considerable interest in linking their project, where appropriate, to our ongoing Delta mercury project. Their additional interest in selenium and potential selenium:mercury interactions, in relation to subsequent bioavailability, links directly to a major hypothesis we are testing for CALFED.

The proposed project is also highly relevant to the extensive mercury-related Directed Action being developed by CALFED at this time, in which our UC Davis research team will work on upstream (Cache Creek watershed) questions of (1) source material relationship to methylation and (2) trophic mercury inter-relationships. A number of other research groups will work on additional important aspects of mercury loading, mass balance, and downstream effects. As a major impetus for this Directed Action is the desire to provide more ecologically relevant input to inevitable future TMDL legislation for mercury, the potential benefits of the proposed project are clear. Development of a novel but reliable new measure of relative sediment contaminant bioavailability that is specifically based on ecological relevance could have widespread application.

The submitting investigators are highly esteemed in the research community. The work they are proposing is not preliminary in nature, but follows on several years of promising initial testing and development. These researchers have clearly passed the selection criteria of numerous Federal and other funding sources for prior and ongoing related work. Their application for transfer of this developing technology to Bay-Delta watershed problems should be considered seriously. We are in strong support of this proposal.

Darell Slotton

Dr. Darell G. Slotton
Dept. of Environmental Science and Policy

Thomas H. Suchanek

Dr. Thomas H. Suchanek
Dept. of Wildlife, Fish, and Conservation Biology

UNIVERSITY OF CALIFORNIA, BERKELEY

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ENVIRONMENTAL
ENGINEERING AND HEALTH SCIENCES
LABORATORY

MAILING ADDRESS:
UNIVERSITY OF CALIFORNIA
1371 S. 46th STREET, BLDG. 112
RICHMOND, CALIFORNIA 94804-4603
(510) 231-9516 FAX (510) 231-5764

April 14, 1999

Dr. Donald Weston
Dept. Integrative Biology
University of California
3060 Valley Life Sciences Bldg.
Berkeley, CA 94720-3140

Dear Dr. Weston:

Thank you for speaking to me about your proposed CALFED project, "A new approach to assess the effect of ecosystem restoration efforts on contaminant bioavailability". I understand that the work includes a field validation task in which you would intend to use your new approach to evaluate the bioavailability of selenium in the pond sludges of our Algal-Bacteria Selenium Removal (ABSR) system. In view of the complex chemistry of selenium, techniques to evaluate selenium bioavailability are of great interest, and I strongly support your efforts. I certainly think it valuable to link your proposed CALFED project with our existing program. You are welcome to collect samples from the ABSR system, and I look forward to seeing your results. If I can help you in any other way please let me know.

Sincerely yours,

William J. Oswald

William J. Oswald, Ph.D.
Professor Emeritus
Environmental Engineering and Public Health

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CENTERS FOR WATER AND WILDLAND RESOURCES
OFFICE OF THE DIRECTOR
(530) 752-8070
FAX: (530) 752-8086
CWWR@ucdavis.edu

ONE SHIELDS AVENUE
DAVIS, CALIFORNIA 95616-8750

Documentation for proposed cost share
2 yr × $26,500/yr = $53,000

April 7, 1999

Professor Donald Weston
Dept. of Integrative Biology
1005 Valley Life Sci Bldg. #1005
University of California
Berkeley, CA 94720

Dear Professor Weston:

I am pleased to inform you that the Water Resources Center Coordinating Board has approved funding for your project entitled *Enhancing the Utility of In Vitro Digestive Fluid Extraction as a Management Tool for Contaminated Aquatic Sediments* beginning July 1, 1999. Your project number is W-927.

Because of the large number of good project proposals submitted, some budgets have been lowered to allow the funding of more projects. The maximum annual budget allocated to any project including yours is $26,500.

You will be contacted later to get a departmental account number to transfer funds into. You will also be asked to report the amounts you want allocated to the various budget categories.

In accepting these funds you are committing yourself to submit an annual progress report by July 15, 2000, July 15, 2001, and a final technical completion report by September 1, 2001. Second year funding will only be allocated if the annual report is submitted on time.

As a matter of policy, we limit travel funds to monies required for the actual conduct of research and funds to support travel to professional meetings where the results of Center sponsored research are being presented. Should you find that you will be presenting the results of this research at a professional meeting during the coming year, you may apply to the Center for a supplemental travel allowance. In applying, you should provide evidence that the travel is for disseminating the results of the research.

Future money may also be available for publication resulting from your research when such publications have been accepted or are actually published. At such time, you may apply to the Center for publication costs.

Congratulations on submitting an excellent proposal.

Sincerely,

Miguel A. Mariño
Interim Associate Director

cc: Department Chair
Contracts and Grants Office

MAM:jw

LOCAL NOTIFICATION

The following letter has been sent to the County Board of Supervisors and the County Planning Department in Contra Costa, Madera, San Joaquin, Solano and Yolo counties.

Dear Sirs:

The University of California, Berkeley is submitting a proposal to CALFED entitled "A New Approach to Assess the Effect of Ecosystem Restoration Efforts on Contaminant Bioavailability". CALFED has requested that all investigators submitting proposals to CALFED notify the Board of Supervisors and Planning Departments in counties in which work will be conducted. This letter serves to provide that notification.

The proposed project is a collaborative effort among investigators at the University of California, University of Maryland, and University of Maine. We are developing a new extraction technique that can be used to determine how much of the mercury or selenium in a sediment sample might be available for bioaccumulation by organisms. Part of the proposed work involves collection of sediments throughout the Delta, Sacramento and San Joaquin Rivers, and it is possible that we may be collecting from water bodies within the boundaries of your county. No work will be done on site other than collection of sediment, and we will transport the sediment to the laboratory for our studies.

CALFED will make the funding decisions later this summer, and if our study is funded, work will be performed from fall 1999 through fall 2002. If you have any questions please call me at 510-231-5626.

Donald P. Weston

The following letter has been sent to the Delta Protection Commission and Bay Conservation and Development Commission.

Dear Sirs:

The University of California, Berkeley is submitting a proposal to CALFED entitled "A New Approach to Assess the Effect of Ecosystem Restoration Efforts on Contaminant Bioavailability". CALFED has requested that all investigators submitting proposals to CALFED notify the Commission of the proposed work. This letter serves to provide that notification.

The proposed project is a collaborative effort among investigators at the University of California, University of Maryland, and University of Maine. We are developing a new extraction technique that can be used to determine how much of the mercury or selenium in a sediment sample might be available for bioaccumulation by organisms. Part of the proposed work involves collection of sediments throughout the Delta, Sacramento and San Joaquin Rivers. No work will be done on site other than collection of sediment, and we will transport the sediment to the laboratory for our studies.

CALFED will make the funding decisions later this summer, and if our study is funded, work will be performed from fall 1999 through fall 2002. If you have any questions please call me at 510-231-5626.

Donald P. Weston

OMB Approval No. 0348-0043

APPLICATION FOR FEDERAL ASSISTANCE

2. DATE SUBMITTED	Application Identifier
3. DATE RECEIVED BY STATE	State Application Identifier
4. DATE RECEIVED BY FEDERAL AGENCY	Federal Identifier

1. TYPE OF SUBMISSION:
Application: ☐ Construction ☒ Non-Construction
Preapplication: ☐ Construction ☐ Non-Construction

5. APPLICATION INFORMATION

Legal Name: The Regents of the University of California

Organizational Unit: University of California, Berkeley; Dept. of Integrative Biology

Address (give city, county, state, and zip code):
University of California, Berkeley
Sponsored Projects Office
336 Sproul Hall, Alameda County
Berkeley, CA 94720-5940

Name and telephone number of the person to be contacted on matters involving this application (give area code)

Administrative Contact: Lynn Deetz (510) 643-6113

Technical Contact: Donald P. Weston, Ph.D. 510-231-5626

6. EMPLOYER IDENTIFICATION NUMBER (EIN): 94-6002123

7. TYPE OF APPLICANT: (enter appropriate letter in box) [I]

A. State
B. County
C. Municipal
D. Township
E. Interstate
F. Intermunicipal
G. Special District
H. Independent School Dist.
I. State Controlled Institution of Higher Learning
J. Private University
K. Indian Tribe
L. Individual
M. Profit Organization
N. Other (Specify):

8. TYPE OF APPLICATION:
☒ New ☐ Continuation ☐ Revision

If Revision, enter appropriate letter(s) in boxes(es) ☐ ☐

A. Increase Award B. Decrease Award C. Increase Duration
D. Decrease Duration Other (specify):

9. NAME OF FEDERAL AGENCY:
CALFED

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER: ☐☐☐ • ☐☐☐

TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT:
A New Approach to Assess the Effects of Ecosystem Restoration Efforts on Contaminant Bioavailability

12. AREAS AFFECTED BY PROJECT (cities, counties, states, etc.)
California

FDP-II

13. PROPOSED PROJECT:

Start Date	Ending Date
10/01/99	9/30/02

14. CONGRESSIONAL DISTRICTS OF:

a. Applicant	b. Project
9th	9th

15. ESTIMATED FUNDING:

a. Federal	$711,773
b. Applicant	$ 53,000
c. State	$
d. Local	$
e. Other	$
f. Program Income	$
g. TOTAL	$764,773

16. IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?
a. YES. THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:
DATE _____
b. NO. ☒ PROGRAM IS NOT COVERED BY E.O. 12372
☐ OR PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. IS THE APPLICANT DELINQUENT ON ANY FEDERAL DEBT?
☐ Yes If "Yes," attach an explanation. ☒ No

18. TO THE BEST OF MY KNOWLEDGE AND BELIEF, ALL DATA IN THIS APPLICATION/PREAPPLICATION ARE TRUE AND CORRECT THE DOCUMENT HAS BEEN DULY AUTHORIZED BY THE GOVERNING BODY OF THE APPLICANT AND THE APPLICANT WILL COMPLY WITH THE ATTACHED ASSURANCES IF THE ASSISTANCE IS AWARDED

a. Typed Name of Authorized Representative	b. Title	c. Telephone number
Lynn Deetz	Senior Research Administrator	(510) 643-6113

d. Signature of Authorized Representative: Lynn E. Deetz C-T-R

e. Date Signed: 4/14/99

Previous Editions Not Usable

Standard Form 424
Prescribed by OMB Circular A-102

Authorized for Local Reproduction

I —018972

I-018972

Donald P. Weston, Ph.D.
University of California, Berkeley

OMB Approval No. 0348-0044

BUDGET INFORMATION – Non-Construction Programs

SECTION A - BUDGET SUMMARY

Grant Program Function or Activity (a)	Catalog of Federal Domestic Assistance Number (b)	Estimated Unobligated Funds		New or Revised Budget		
		Federal (c)	Non-Federal (d)	Federal (e)	Non-Federal (f)	Total (g)
1. Ecosystem Restoration Program		$	$	$ 711,773	$ 53,000	$ 764,773
2.						
3.						
4.						
5. TOTALS		$	$	$ 711,773	$ 53,000	$ 764,773

SECTION B - BUDGET CATEGORIES

6. Object Class Categories	GRANT PROGRAM, FUNCTION OR ACTIVITY (1) Total Project	(2)	(3)	(4)	(5)	TOTAL (6)
a. Personnel	88,649	0	0	0	0	88,649
b. Fringe Benefits	13,858	0	0	0	0	13,858
c. Travel	8,400	0	0	0	0	8,400
d. Equipment	35,000	0	0	0	0	35,000
e. Supplies	27,300	0	0	0	0	27,300
f. Contractual	429,571	0	0	0	0	429,571
g. Construction	0	0	0	0	0	0
h. Other	9,400	0	0	0	0	9,400
i. Total Direct Charges (Sum of 6a - 6h)	612,178	0	0	0	0	612,178
j. Indirect Charges	99,595	0	0	0	0	99,595
k. TOTALS (Sum of 6i and 6j)	$ 711,773	$ -	$ -	$ -	$ -	$ 711,773
7. Program Income	$	$	$	$	$	$

I—018973

I-018973

Donald P. Weston, Ph.D.
University of California, Berkeley

SECTION C - NON-FEDERAL RESOURCES

(a) Grant Program	(b) Applicant	(c) State	(d) Other Sources	(e) TOTALS
8. Ecosystem Restoration Program	$ 53,000	$	$	$ 53,000
9.				
10.				
11.				
12. TOTALS (sum of lines 8 and 11)	$ 53,000	$	$	$ 53,000

SECTION D - FORECASTED CASH NEEDS

	Total for 1st Year	1st Quarter	2nd Quarter	3rd Quarter	4th Quarter
13. Federal	$ 272,215	$ 68,439	$ 62,449	$ 86,363	$ 54,964
14. NonFederal					
15. TOTALS (sum of lines 13 and 14)	$ 272,215	$ 68,439	$ 62,449	$ 86,363	$ 54,964

SECTION E- BUDGET ESTIMATES OF FEDERAL NEEDS FOR BALANCE OF THE PROJECT

(a) Grant Program	FUTURE FUNDING PERIODS (Years) (b) First	(c) Second	(d) Third	(e) Fourth	(f) Fifth
16. Ecosystem Restoration Program	$ 272,215	$ 237,437	$ 202,121		
17.					
18.					
19.					
20. TOTALS (sum of lines 16 - 19)	$ 272,215	$ 237,437	$ 202,121		

SECTION F - OTHER BUDGET INFORMATION

(Use Additional Sheets If Necessary)

21. Direct Charges: $612,178

22. Indirect Charges: $99,595

23. Remarks:

Indirect Costs are calculated at a rate of 50.4% of Modified Total Direct Costs (i.e., less equipment & less subcontract amounts over initial $25,000).

Agency

State of California DEPARTMENT OF WATER RESOURCES The Resources Agency

Agreement No. _____

Exhibit _____

STANDARD CLAUSES -
INTERAGENCY AGREEMENTS

Audit Clause. For contracts in excess of $10,000, the contracting parties shall be subject to the examination and audit of the State Auditor for a period of three years after final payment under the contract. (Government Code Section 8546.7).

Availability of Funds. Work to be performed under this contract is subject to availability of funds through the State's normal budget process.

Interagency Payment Clause. For services provided under this agreement, charges will be computed in accordance with State Administrative Manual Section 8752 and 8752.1.

Termination Clause. Either State agency may terminate this contract upon 30 days advance written notice. The State agency providing the services shall be reimbursed for all reasonable expenses incurred up to the date of termination.

Severability. If any provision of this Agreement is held invalid or unenforceable by any court of final jurisdiction, it is the intent of the parties that all other provisions of this Agreement be construed to remain fully valid, enforceable, and binding on the parties.

Y2K Language. The Contractor warrants and represents that the goods or services sold, leased, or licensed to the State of California, its agencies, or its political subdivisions, pursuant to this Agreement are "Year 2000 compliant" For purposes of this Agreement, a good or service is year 2000 compliant if it will continue to fully function before, at, and after the Year 2000 without interruption and, if applicable, with full ability to accurately and unambiguously process, display, compare, calculate, manipulate, and otherwise utilize date information. This warranty and representation supersedes all warranty disclaimers and limitations and all limitations on liability provided by or through the Contractor.

DWR 4187 (REV. 1/99)

Certifications Regarding Debarment, Suspension and Other Responsibility Matters, Drug-Free Workplace Requirements and Lobbying

Persons signing this form should refer to the regulations referenced below for complete instructions:

Certification Regarding Debarment, Suspension, and Other Responsibility Matters - Primary Covered Transactions - The prospective primary participant further agrees by submitting this proposal that it will include the clause titled, "Certification Regarding Debarment, Suspension, Ineligibility and Voluntary Exclusion - Lower Tier Covered Transaction," provided by the department or agency entering into this covered transaction, without modification, in all lower tier covered transactions and in all solicitations for lower tier covered transactions. See below for language to be used; use this form for certification and sign; or use Department of the Interior Form 1954 (DI-1954). (See Appendix A of Subpart D of 43 CFR Part 12.)

Certification Regarding Debarment, Suspension, Ineligibility and Voluntary Exclusion - Lower Tier Covered Transactions - (See Appendix B of Subpart D of 43 CFR Part 12.)

Certification Regarding Drug-Free Workplace Requirements - Alternate I. (Grantees Other Than Individuals) and Alternate II. (Grantees Who are Individuals) - (See Appendix C of Subpart D of 43 CFR Part 12)

Signature on this form provides for compliance with certification requirements under 43 CFR Parts 12 and 18. The certifications shall be treated as a material representation of fact upon which reliance will be placed when the Department of the Interior determines to award the covered transaction, grant, cooperative agreement or loan.

PART A: Certification Regarding Debarment, Suspension, and Other Responsibility Matters - Primary Covered Transactions

CHECK__IF THIS CERTIFICATION IS FOR A PRIMARY COVERED TRANSACTION AND IS APPLICABLE.

(1) The prospective primary participant certifies to the best of its knowledge and belief, that it and its principals:

(a) Are not presently debarred, suspended, proposed for debarment, declared ineligible, or voluntarily excluded from covered transactions by any Federal department or agency;

(b) Have not within a three-year period preceding this proposal been convicted of or had a civil judgment rendered against them for commission of fraud or a criminal offense in connection with obtaining, attempting to obtain, or performing a public (Federal, State or local) transaction or contract under a public transaction; violation of Federal or State antitrust statutes or commission of embezzlement, theft, forgery, bribery, falsification or destruction of records, making false statements, or receiving stolen property;

(c) Are not presently indicted for or otherwise criminally or civilly charged by a governmental entity (Federal, State or local) with commission of any of the offenses enumerated in paragraph (1)(b) of this certification; and

(d) Have not within a three-year period preceding this application/proposal had one or more public transactions (Federal, State or local) terminated for cause or default.

(2) Where the prospective primary participant is unable to certify to any of the statements in this certification, such prospective participant shall attach an explanation to this proposal.

PART B: Certification Regarding Debarment, Suspension, Ineligibility and Voluntary Exclusion - Lower Tier Covered Transactions

CHECK__IF THIS CERTIFICATION IS FOR A LOWER TIER COVERED TRANSACTION AND IS APPLICABLE.

(1) The prospective lower tier participant certifies, by submission of this proposal, that neither it nor its principals is presently debarred, suspended, proposed for debarment, declared ineligible, or voluntarily excluded from participation in this transaction by any Federal department or agency.

(2) Where the prospective lower tier participant is unable to certify to any of the statements in this certification, such prospective participant shall attach an explanation to this proposal.

DI-2010
March 1995
(This form consolidates DI-1953, DI-1954, DI-1955, DI-1956 and DI-1962)

PART C: Certification Regarding Drug-Free Workplace Requirements

CHECK__IF THIS CERTIFICATION IS FOR AN APPLICANT WHO IS NOT AN INDIVIDUAL.

Alternate I. (Grantees Other Than Individuals)

A. The grantee certifies that it will or continue to provide a drug-free workplace by:

(a) Publishing a statement notifying employees that the unlawful manufacture, distribution, dispensing, possession, or use of a controlled substance is prohibited in the grantee's workplace and specifying the actions that will be taken against employees for violation of such prohibition;

(b) Establishing an ongoing drug-free awareness program to inform employees about--
(1) The dangers of drug abuse in the workplace;
(2) The grantee's policy of maintaining a drug-free workplace;
(3) Any available drug counseling, rehabilitation, and employee assistance programs; and
(4) The penalties that may be imposed upon employees for drug abuse violations occurring in the workplace;

(c) Making it a requirement that each employee to be engaged in the performance of the grant be given a copy of the statement required by paragraph (a);

(d) Notifying the employee in the statement required by paragraph (a) that, as a condition of employment under the grant, the employee will –
(1) Abide by the terms of the statement; and
(2) Notify the employer in writing of his or her conviction for a violation of a criminal drug statute occurring in the workplace no later than five calendar days after such conviction;

(e) Notifying the agency in writing, within ten calendar days after receiving notice under subparagraph (d)(2) from an employee or otherwise receiving actual notice of such conviction. Employers of convicted employees must provide notice, including position title, to every grant officer on whose grant activity the convicted employee was working, unless the Federal agency has designated a central point for the receipt of such notices. Notice shall include the identification numbers(s) of each affected grant;

(f) Taking one of the following actions, within 30 calendar days of receiving notice under subparagraph (d)(2), with respect to any employee who is so convicted –
(1) Taking appropriate personnel action against such an employee, up to and including termination, consistent with the requirements of the Rehabilitation Act of 1973, as amended; or
(2) Requiring such employee to participate satisfactorily in a drug abuse assistance or rehabilitation program approved for such purposes by a Federal, State, or local health, law enforcement, or other appropriate agency;

(g) Making a good faith effort to continue to maintain a drug-free workplace through implementation of paragraphs (a) (b), (c), (d), (e) and (f).

B. The grantee may insert in the space provided below the site(s for the performance of work done in connection with the specific grant:

Place of Performance (Street address, city, county, state, zip code)

Check___if there are workplaces on file that are not identified here.

PART D: Certification Regarding Drug-Free Workplace Requirements

CHECK__IF THIS CERTIFICATION IS FOR AN APPLICANT WHO IS AN INDIVIDUAL.

Alternate II. (Grantees Who Are Individuals)

(a) The grantee certifies that, as a condition of the grant, he or she will not engage in the unlawful manufacture, distribution, dispensing, possession, or use of a controlled substance in conducting any activity with the grant;

(b) If convicted of a criminal drug offense resulting from a violation occurring during the conduct of any grant activity, he or she will report the conviction, in writing, within 10 calendar days of the conviction, to the grant officer or other designee, unless the Federal agency designates a central point for the receipt of such notices. When notice is made to such a central point, it shall include the identification number(s) of each affected grant

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PART E: **Certification Regarding Lobbying**
Certification for Contracts, Grants, Loans, and Cooperative Agreements

CHECK__ IF CERTIFICATION IS FOR THE AWARD OF ANY OF THE FOLLOWING AND THE AMOUNT EXCEEDS $100,000: A FEDERAL GRANT OR COOPERATIVE AGREEMENT; SUBCONTRACT, OR SUBGRANT UNDER THE GRANT OR COOPERATIVE AGREEMENT.

CHECK__ IF CERTIFICATION IS FOR THE AWARD OF A FEDERAL LOAN EXCEEDING THE AMOUNT OF $150,000, OR A SUBGRANT OR SUBCONTRACT EXCEEDING $100,000, UNDER THE LOAN.

The undersigned certifies, to the best of his or her knowledge and belief, that:

(1) No Federal appropriated funds have been paid or will be paid, by or on behalf of the undersigned, to any person for influencing or attempting to influence an officer or employee of an agency, a Member of Congress, and officer or employee of Congress, or an employee of a Member of Congress in connection with the awarding of any Federal contract, the making of any Federal grant, the making of any Federal loan, the entering into of any cooperative agreement, and the extension, continuation, renewal, amendment, or modification of any Federal contract, grant, loan, or cooperative agreement.

(2) If any funds other than Federal appropriated funds have been paid or will be paid to any person for influencing or attempting to influence an officer or employee of any agency, a Member of Congress, an officer or employee of Congress, or an employee of a Member of Congress in connection with this Federal contract, grant, loan, or cooperative agreement, the undersigned shall complete and submit Standard Form-LLL, "Disclosure Form to Report Lobbying," in accordance with its instructions.

(3) The undersigned shall require that the language of this certification be included in the award documents for all subawards at all tiers (including subcontracts, subgrants, and contracts under grants, loans, and cooperative agreements) and that all subrecipients shall certify accordingly.

This certification is a material representation of fact upon which reliance was placed when this transaction was made or entered into. Submission of this certification is a prerequisite for making or entering into this transaction imposed by Section 1352, title 31, U.S. Code. Any person who fails to file the required certification shall be subject to a civil penalty of not less than $10,000 and not more than $100,000 for each such failure.

As the authorized certifying official, I hereby certify that the above specified certifications are true.

SIGNATURE OF AUTHORIZED CERTIFYING OFFICIAL Lynn E. Deetz C.T.A.

TYPED NAME AND TITLE Lynn E. Deetz
Senior Research Administrator

DATE 4-14-99

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OMB Approval No. 0348-0040

ASSURANCES - NON-CONSTRUCTION PROGRAMS

Public reporting burden for this collection of information is estimated to average 15 minutes per response, including time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding the burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to the Office of Management and Budget, Paperwork Reduction Project (0348-0040), Washington, DC 20503.

PLEASE DO NOT RETURN YOUR COMPLETED FORM TO THE OFFICE OF MANAGEMENT AND BUDGET. SEND IT TO THE ADDRESS PROVIDED BY THE SPONSORING AGENCY.

NOTE: Certain of these assurances may not be applicable to your project or program. If you have questions, please contact the awarding agency. Further, certain Federal awarding agencies may require applicants to certify to additional assurances. If such is the case, you will be notified.

As the duly authorized representative of the applicant, I certify that the applicant:

1. Has the legal authority to apply for Federal assistance and the institutional, managerial and financial capability (including funds sufficient to pay the non-Federal share of project cost) to ensure proper planning, management and completion of the project described in this application.

2. Will give the awarding agency, the Comptroller General of the United States and, if appropriate, the State, through any authorized representative, access to and the right to examine all records, books, papers, or documents related to the award; and will establish a proper accounting system in accordance with generally accepted accounting standards or agency directives.

3. Will establish safeguards to prohibit employees from using their positions for a purpose that constitutes or presents the appearance of personal or organizational conflict of interest, or personal gain.

4. Will initiate and complete the work within the applicable time frame after receipt of approval of the awarding agency.

5. Will comply with the Intergovernmental Personnel Act of 1970 (42 U.S.C. §§4728-4763) relating to prescribed standards for merit systems for programs funded under one of the 19 statutes or regulations specified in Appendix A of OPM's Standards for a Merit System of Personnel Administration (5 C.F.R. 900, Subpart F).

6. Will comply with all Federal statutes relating to nondiscrimination. These include but are not limited to: (a) Title VI of the Civil Rights Act of 1964 (P.L. 88-352) which prohibits discrimination on the basis of race, color or national origin; (b) Title IX of the Education Amendments of 1972, as amended (20 U.S.C. §§1681-1683, and 1685-1686), which prohibits discrimination on the basis of sex; (c) Section 504 of the Rehabilitation Act of 1973, as amended (29 U.S.C. §794), which prohibits discrimination on the basis of handicaps; (d) the Age Discrimination Act of 1975, as amended (42 U.S.C. §§6101-6107), which prohibits discrimination on the basis of age; (e) the Drug Abuse Office and Treatment Act of 1972 (P.L. 92-255), as amended, relating to nondiscrimination on the basis of drug abuse; (f) the Comprehensive Alcohol Abuse and Alcoholism Prevention, Treatment and Rehabilitation Act of 1970 (P.L. 91-616), as amended, relating to nondiscrimination on the basis of alcohol abuse or alcoholism; (g) §§523 and 527 of the Public Health Service Act of 1912 (42 U.S.C. §§290 dd-3 and 290 ee 3), as amended, relating to confidentiality of alcohol and drug abuse patient records; (h) Title VIII of the Civil Rights Act of 1968 (42 U.S.C. §§3601 et seq.), as amended, relating to nondiscrimination in the sale, rental or financing of housing; (i) any other nondiscrimination provisions in the specific statute(s) under which application for Federal assistance is being made; and, (j) the requirements of any other nondiscrimination statute(s) which may apply to the application.

7. Will comply, or has already complied, with the requirements of Titles II and III of the Uniform Relocation Assistance and Real Property Acquisition Policies Act of 1970 (P.L. 91-646) which provide for fair and equitable treatment of persons displaced or whose property is acquired as a result of Federal or federally-assisted programs. These requirements apply to all interests in real property acquired for project purposes regardless of Federal participation in purchases.

8. Will comply, as applicable, with provisions of the Hatch Act (5 U.S.C. §§1501-1508 and 7324-7328) which limit the political activities of employees whose principal employment activities are funded in whole or in part with Federal funds.

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9. Will comply, as applicable, with the provisions of the Davis-Bacon Act (40 U.S.C. §§276a to 276a-7), the Copeland Act (40 U.S.C. §276c and 18 U.S.C. §874), and the Contract Work Hours and Safety Standards Act (40 U.S.C. §§327-333), regarding labor standards for federally-assisted construction subagreements.

10. Will comply, if applicable, with flood insurance purchase requirements of Section 102(a) of the Flood Disaster Protection Act of 1973 (P.L. 93-234) which requires recipients in a special flood hazard area to participate in the program and to purchase flood insurance if the total cost of insurable construction and acquisition is $10,000 or more.

11. Will comply with environmental standards which may be prescribed pursuant to the following: (a) institution of environmental quality control measures under the National Environmental Policy Act of 1969 (P.L. 91-190) and Executive Order (EO) 11514; (b) notification of violating facilities pursuant to EO 11738; (c) protection of wetlands pursuant to EO 11990; (d) evaluation of flood hazards in floodplains in accordance with EO 11988; (e) assurance of project consistency with the approved State management program developed under the Coastal Zone Management Act of 1972 (16 U.S.C. §§1451 et seq.); (f) conformity of Federal actions to State (Clean Air) Implementation Plans under Section 176(c) of the Clean Air Act of 1955, as amended (42 U.S.C. §§7401 et seq.); (g) protection of underground sources of drinking water under the Safe Drinking Water Act of 1974, as amended (P.L. 93-523); and, (h) protection of endangered species under the Endangered Species Act of 1973, as amended (P.L. 93-205).

12. Will comply with the Wild and Scenic Rivers Act of 1968 (16 U.S.C. §§1271 et seq.) related to protecting components or potential components of the national wild and scenic rivers system.

13. Will assist the awarding agency in assuring compliance with Section 106 of the National Historic Preservation Act of 1966, as amended (16 U.S.C. §470), EO 11593 (identification and protection of historic properties), and the Archaeological and Historic Preservation Act of 1974 (16 U.S.C. §§469a-1 et seq.).

14. Will comply with P.L. 93-348 regarding the protection of human subjects involved in research, development, and related activities supported by this award of assistance.

15. Will comply with the Laboratory Animal Welfare Act of 1966 (P.L. 89-544, as amended, 7 U.S.C. §§2131 et seq.) pertaining to the care, handling, and treatment of warm blooded animals held for research, teaching, or other activities supported by this award of assistance.

16. Will comply with the Lead-Based Paint Poisoning Prevention Act (42 U.S.C. §§4801 et seq.) which prohibits the use of lead-based paint in construction or rehabilitation of residence structures.

17. Will cause to be performed the required financial and compliance audits in accordance with the Single Audit Act Amendments of 1996 and OMB Circular No. A-133, "Audits of States, Local Governments, and Non-Profit Organizations."

18. Will comply with all applicable requirements of all other Federal laws, executive orders, regulations, and policies governing this program.

SIGNATURE OF AUTHORIZED CERTIFYING OFFICIAL	TITLE
Lynn E. Daetz	Lynn E. Daetz Senior Research Administrator
APPLICANT ORGANIZATION	DATE SUBMITTED
THE REGENTS OF THE UNIVERSITY OF CALIFORNIA	4-14-98

Standard Form 424B (Rev. 7-97) Ba

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