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F1-022

Endocrine Disrupting Chemicals in the San Francisco Bay-Estuary Catchment

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Proposal

Submitted to

CALFED Bay-Delta Program Office
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by

Applied
Sciences

July 28, 1997

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I-000732

I-000732

I. EXECUTIVE SUMMARY

a. Project Title and Applicant Names:"Endocrine disrupting chemicals in the San Francisco Bay-Estuary catchment," submitted by Robert B. Spies, Applied Marine Sciences, Inc.; Peter Thomas, University of Texas; Scott Ogle, Pacific Eco-Risk Laboratories; and David Dodge, McClaren-Hart Engineers.

b. Project Description and Ecological Objectives: To determine if populations of declining fish are affected by endocrine-disrupting chemicals in the catchment of the San Francisco Bay-Estuary.

c. **Approach/Tasks/Schedule**:

1. Approach: Water samples will be collected from 20 areas within the major Rivers and Delta at places and times that represent both background conditions and known sources of chemical contamination We will assay the water samples for endocrine-disrupting activity with fathead minnows. This is a surrogate species for larger fish in the ecosystem that are more difficult and expensive to breed under controlled conditions. The assays are carried out under carefully defined conditions and last 3 weeks. At the end of the assays minnows from treatments and controls will be compared for a variety of endocrine-mediated effects, including sex reversal, inhibition of egg production, induction of egg yolk in males, and plasma steroids.
2. Tasks/schedule:

(a). Collection of water samples: 11/97-5/98; Reproductive assays: 11/97-6/98; endocrine measurements: 12/98-7/98; analyze data: 8/98; Write report: 9/98.

d. Justification: Many species of fish, including Delta smelt, splittail, striped bass, various runs of Chinook salmon and green sturgeon, are declining in the system (Herboldt, 1992). Although some of these declines appear to be related to the amount of freshwater flowing through the system, the exact mechanisms of effect are not known and contaminants could have a role in some declines. Contaminants can affect reproduction in fish by mimicking hormones, either speeding up, slowing down or completely inhibiting certain portions of the reproductive cycle. Very little work on reproductive effects of contaminants has been done in the system, but there is strong correlative evidence that contaminates are affecting some species in the Bay-Delta system. This proposed work is an important component of testing the null hypothesis that contaminants have no role in declines in fish populations in the system. If CALFED is to restore fish populations in the system it needs to know if this can be accomplished at least in part by reducing inputs of endocrine-disrupting chemicals.

e. **Budget: $99K**

f. Applicant qualifications: Dr. Robert B. Spies is a nationally known aquatic toxicologist and ecologist that first described a link between organic contaminants and reproductive problems in San Francisco Bay; Dr. Peter Thomas is one of the country's foremost experts on the effects of contaminants on fish reproduction.

g. Monitoring And Data Evaluation: Results of this study may indicate parameters for incorporating into existing monitoring programs.

h. Local Support/Coordination with Other Programs/Compatibility with CALFED Objectives: This project will be coordinated with existing contaminant studies through the PWT working group.

II. TITLE PAGE

Endocrine disrupting chemicals in the San Francisco Bay-Estuary catchment

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750 Channel View Drive
Port Aransas, Texas 78373

Scott Ogle, Ph.D.
Pacific Eco-Risk
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Martinez, CA 94553

David Dodge
McClaren-Hart Engineers.
1135 Atlantic Avenue
Alameda, California 94501

Project group: Other Services

Submitted for CALFED funding
July 28, 1997

III. PROJECT DESCRIPTION

a. Project Description and Approach: This proposal is an initial step in determining if exotic chemicals in the ecosystem have effects on reproduction in fish. We propose to assay fresh waters in the catchment of the system to determine their potential for inducing endocrine changes and alteration of reproduction in fish. We will do this with an established assay system utilizing fathead minnows (Kramer et al., in press). Contaminants in the system have the potential for altering the timing of reproduction, sex of the fish, and inducing egg-yolk in male fish.

(1) *Specific areas to be surveyed*--We will survey 10 sites in the Sacramento and San Joaquin Rivers and the Delta where the salinity is no greater than 2 parts per thousand, the upper limit of tolerance for our assay species, the fathead minnow. The sites are chosen to represent both sources of potential contaminants, such as agricultural runoff, sewage discharge and general background conditions in various reaches of the system, such as the mainstem Sacramento and San Joaquin Rivers. Proposed sampling sites are listed in Table 1. This selection of sites may change as new information becomes available, but the goal of assaying background conditions and some major sources is the principle that we will employ.

Table 1. Candidate sampling sites

Sacramento River Mainstem, Redding
Sacramento River Mainstem, below Redding
Sacramento River. at Colusa Drain
Sacramento Slough
Sacramento River, POTW outfall
Stockton Turning Basin
Prospect Slough
Orestimo Creek
Turlock Irrigation District, Lateral 5
San Joaquin River, Mainstem

(2) Assay system--Fathead minnows can be manipulated to induce spawning. We will compare spawning success and related measures of fish in control water with that of fish in assay water (charcoal-filtered spring water). All fish will be acclimated for 3 weeks prior to assay. For each assay 3 males and 3 females will be placed in a 19-L glass aquarium. The aquaria will be aerated. Fish will be fed at a rate of 0.5% body weight/day. Initial conditions will be a water temperature of 19°C; 16h light:9h dark. Water temperature will be raised 2.3°C/week during acclimation to a final of 26°C, the optimal breeding temperature. Water will be changed weekly. At the initiation of the assays, fish will be held in the treatment water for 6 days without breeding tiles (strongly discourages breeding) in order to react to any adverse chemicals in the water and then provided with the tiles during two subsequent 6-day periods (with 1 day rest between). Breeding tiles, which are small

flower pots cut in half lengthwise, will be checked twice daily and the number of eggs counted and photographed. Any tiles with eggs will be removed and replaced immediately with fresh tiles. Fish will be sacrificed at the end of the 3-week period and a capillary blood sample taken. Triplicate analyses will be carried out on water from each site.

(3) Assessing effects--Endocrine disrupting chemicals can cause acute toxicity (Kramer et al, in press) and some ambient water samples from the catchment have been toxic to the fathead minnows (Fox, 1996). Therefore, we will track both male and female survival, recording any deaths that occur on a daily basis during the assays. Contaminants can cause a reduction in spawning or fecundity (e.g., Holm et al., 1973; Masek, 1968), therefore eggs will be counted daily and the data will be evaluated as the timing of spawning, spawning success and mean number of eggs produced per female in each treatment. Subtler endocrine effects will be evaluated through determination of plasma concentrations of estradiol and vitellogenin, which can be affected by contaminants (e.g., Spies and Thomas, in press). Also, environmental xenoestrogens (=some contaminants) can induce egg yolk proteins in male fish (e.g., Harries et al., 1996).

(4) Data analysis--All of the above experimental variables will be analyzed by non-parametric analyses: Mann-Whitney U test for two-group analyses; Spearman rank correlation for correlations, and Kruskall-Wallis for one-way analyses of variance.

(5) Interpretation--The results of tests will be considered significant if the probability of significance is greater than 95% (alpha>0.05). Since this is an initial survey for the area, the ultimate ecological significance, as separate from the statistical significance of the results, cannot be attained without further work. However, widespread effects or effects that indicate potential susceptibility of some species, by nature of the location of spawning populations nearby at the time of water collection, would point the way to further specific investigations, including identification of the chemicals responsible for the observed effects.

b. **Location of the Project:** This project will take samples at the locations indicated in Table 1, which are meant to represent most of the entire catchment in CALFED's preview.

c. **Expected Benefits:** If chemicals in the catchment of the system are having widespread effects on the reproduction of fish, this survey will likely identify the initial dimensions of the problem and point the way to further testing that would pinpoint the places, times and chemicals that would be responsible. Steps could then be taken to control or mitigate the effects of such chemicals.

d. **Background and Biological/Technical Justification:** A significant number of fish populations in the San Francisco Bay-Delta system have declined in recent years, including winter-run Chinook salmon (*Onchorhynchus tshawytscha*), the striped bass (*Morone saxatilis*), the Sacramento Splittail (*Pogonichthys macrolepidotus*), the Longfin Smelt (*Spirinchus thaleichthys*), the Delta Smelt (*Hypomesus transpacificus*), Steelhead (*Onchorhychus mykiss*), sturgeon (*Acipenser* spp). and

surfperch (*Embiotocidae*) (Herbold et al., 1992). The reasons for these declines are unclear, as there are numerous anthropogenic factors (dyking, water diversions, etc.) and decadal-scales of climatic change (e.g., see Foe, 1995; Moyle et. al, 1992; Laevastu, 1993). Water flow is correlated with several population fluctuations, but the mechanisms that link such flow to healthy populations (e.g., better food production, greater contaminant dilution, etc.) and the role that other factors may play is not clear. In order for CALFED to restore fish populations in the system, a better understanding of the causes of these declines is needed.

It is clear that toxic lethal concentrations of contaminants exist at certain times and places in the system (Ogle, 1996; Fox, unpublished), which would suggest that sublethal effects (e.g., reproductive) probably exist and may be widespread.

Contaminants may be affecting fish populations in the system, since the Bay-Delta ecosystem receives a large variety of contaminants (Gunther et al., 1987; Davis et al., 1992), including significant quantities of pesticides and metals and their carriers, which are used in surrounding agricultural lands and suburban landscapes, particularly in the Delta (e.g., Menconi and Cox, 1994). Many of these contaminants can disrupt endocrine function or otherwise be toxic to embryos, larvae and juveniles of fish. The highest use of pesticides occurs in the winter and spring, when most of the above species reproduce (Adams et al., 1996). Also, a study of starry flounder (*Platichthys flesus*) conducted in the mid-1980s showed that contaminants are strongly linked to poor quality eggs and reproductive impairment in the central Bay (Spies and Rice, 1988). In addition, a recent study has pointed to alterations of sex steroid concentrations in carp that correlate with dissolved pesticides in US river waters, including a strong hint that effects may occur in the SF Bay-Delta (Goodbred et al., 1997). Any reproductive effects are probably mediated through endocrine disruption and may be widespread, but there has been little follow up work that would indicate how pervasive such effects are in the system, i.e. in other species.

Although the last several years has seen great advances in our understanding of the distribution and abundance of contaminants in the estuary (e.g., SFEI, 1995), there has not been as much emphasis on the risk they might pose to the health of individuals and populations in the ecosystem as a whole. Defining the average chemical contaminant field, as has occurred through implementation of the Regional Monitoring Program (PMP) by the San Francisco Estuary Institute, is a large step forward in understanding risk to the fauna and flora. To complete the risk paradigm, greater efforts towards understanding sublethal toxicity are needed. This proposal is directed towards understanding reproductive risks from contaminants in the catchment of the estuary.

Contaminants can alter endocrine function in wild populations of fish (Rolland et. al, 1997), and such alterations may be expressed as poor egg quality, reduced fecundity, inhibition of spawning, and reduced survival of eggs through hatching. Some overall assessment of the potential for conditions in the system to affect

reproduction of fish is needed as a step towards rejecting the hypothesis that contaminants are important factors affecting some populations of fish.

References/Supporting Documentation

Adams, W., L. Davis, J. Gidings, L. Hall, Jr., R. Smith, K. Solomon and D. Vogel. 1996. An ecological risk assessment of diazinon in the Sacramento and San Joaquin River Basins. Prepared for Ciba-Geigy Corporation, Ciba Crop Protection, Greensboro, North Carolina.

Davis, J.A., A.J. Gunther and J.M. O'Connor. 1992. Priority pollutant loads from effluent discharges to the San Francisco Estuary. Water Environment Research. 64:134–140.

Foe, C. 1995. Evaluation of the potential impact of contaminants on aquatic resources in the Central Valley and Sacramento-San Joaquin Delta Estuary. June 1995. Central Valley Regional Water Quality Control Board. Sacramento, CA.

Fox, P. 1996. Fathead minnow toxicity in the Sacramento River watershed. A PWT oral report and notes.

Goodbred, S.L., R.J. Gilliom, T.S. Gross, N.P. Denslow, W.L. Bryant and T.R. Schoeb. 1997. Reconnaissance of 17ß-estradiol, 11-ketotestosterone, vitellogenin, and gonad histopathology in common carp of the United States: potential for contaminant-induced endocrine disruption. U.S. Geol. Survey, Sacramento, CA. Open-File report 96-627, 47 pp.

Gunther, A. J., J.A. Davis and D.J.H. Phillips. 1987. An Assessment of The Loading of Toxic Contaminants to The San Francisco Bay–Delta. Prepared for The San Francisco Estuary Project, USEPA, and The State Water Resources Control Board of California by the San Francisco Estuary Institute, 330 pp.

Harries, J.E., D.A. Sheahan, S. Jobling, P. Mathiessen P. Neall, E.J. Routledge, R. Rycroft, J.P. Sumpter and T. Taylor. 1996. A survey of estrogenic activity in United Kingdom inland waters. Environ. Toxicol. Chem. 15: 1993-2002.

Herbold, B., A.D. Jassby and P.B. Moyle. 1992. Status and Trends Report on aquatic resources in the San Francisco Bay Estuary. San Francisco Project. PO Box 2050, Oakland, CA 94604-2050, 257 pp.

Holm, G.L., L. Norgren, Y. Anderson and A. Thuren. 1993. effects of esxposure to food contaminated with PBDE, PCN or PCB on reproduction, liver morphology and cytochrome P450 activity in the three-spined stickleback, *Gasteroseus aculeatus*. Aquatic Toxicology 27: 33-50.

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exposure, in fathead minnows (*Pimephales promelas*) exposed to waterborne 17ß-estradiol. In Press: Aquatic Toxicol.

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Macek, K.J. 1968. Reproduction in brook trout (Salvelinus fontinalis) fed sublethal concentrations of DDT. J. Fish. Res. Bd. Canada 25: 1787-1796.

Menconi, M. and C. Cox. 1994. Hazard assessment of the insecticide diazinon to aquatic organisms in the Sacramento-San Joaquin river system. Administrative Report 94-2, California Department of Fish and Game, Rancho Cordova, CA.

Moyle, P.B., B. Herbold, D.E. Stevens and L.W. Miller, 1992. Life history and status of the Delta Smelt in the Sacramento-San Joaquin River estuary in California. Trans. Am. Fish. Soc. 121, 67-77.

Ogle, S., J. Costifas, C. Foe, V. Connor, L. Deanovik, T. Kimball and E. Reyes. 1996. A preliminary survey of sediment toxicity in California's Central Valley. PWT Report from Pacific Eco-risk Laboratories, Central Valley Regional Water Quality Control Board and University of California. Davis, 24 pp.

Rolland RM, Gilbertson M, Peterson RE, eds. "Chemically induced alterations in functional development and reproduction of fishes." SETAC Press, Pensacola FL. (In Press).

San Francisco Estuary Institute. 1995. San Francisco Estuary Regional Monitoring Program for Trace Substances, 1994 Annual Report. San Francisco Estuary Institute, Richmond, CA. 339 pp.

Spies, R.B. and D.W. Rice, Jr. 1988. Effects of organic contaminants on reproduction of the starry flounder *Platichthys stellatus* in San Francisco Bay. II. Reproductive success of fish captured in San Francisco Bay and spawned in the laboratory. Mar. Biol. 98, 191-200.

Spies, R.B., J.J. Stegeman, D.E. Hinton, B. Woodin, R. Smolowitz, M. Okihiro and D. Shea. 1996. Biomarkers of hydrocarbon exposure and sublethal effects in embiotocid fishes from a natural petroleum seep in the Santa Barbara Channel. Aquat. Toxicol. 34, 195-219.

Spies, R.B. and P, Thomas. Reproductive and endocrine status of mature female kelp bass Paralabrax clathratus from a contaminated site in the Southern California Bight and estrogen receptor binding of DDTs, Chapter 9, in Rolland R.M., Gilbertson, M., Peterson R.E.,(eds.) *Chemically induced alterations in functional development and reproduction of fishes*. SETAC Press, Pensacola FL. (In Press).

e. **Proposed Scope of Work**: The following steps/tasks constitute the work:

(1) Finalize sampling sites and times by conferring with EIP/PWT working groups; (2) Collect water samples; (3) Carry out water assays (at Pacific Eco-Risk, Martinez); (4) Sacrifice fish, take plasma samples; (5) Analysis of plasma samples for ALP, estrogen; (6) Data analysis; (7) Report writing and submittal; and (8) Submission of article to peer-reviewed technical journal.

f. **Monitoring and data evaluation**: It is too early to determine if any of the proposed measures might constitute part of an ongoing monitoring program, but every attempt will be made (through the PWT network) to coordinate this work with other programs, such as EIP and the USGS work on pesticides in Delta waters. This current proposal has been developed while the author was a participant in the PWT group that has met regularly during 1996 and 1997. The concept of investigating endocrine disruption was favorably received by the group and the selection of stations presented here was developed with several members of the group. I would urge that this proposal receive independent peer review by CALFED . Hopefully, such a process will be established for the first round of proposals for Category III funding.

g. **Implementability:** This project will be easily implemented, since it requires no permits and will be in compliance with all state, federal and local laws. We are greatly in favor of public participation and would like to have advice from CALFED as to whether any specific actions in this areas would be appropriate if we are funded.

IV. COSTS AND SCHEDULES

a. Budget costs (See Table 2)

1) Contract Justification

a) Dr. Thomas and Dr. Spies have worked together on several projects investigating reproductive effects in fish. Dr. Thomas is needed for interpretation of the endocrinological data.

b) McClaren-Hart Englineers is needed to provide additional manpower in the field effort.

c) Pacific Eco-Risk Laboratory is very experiences with fathead minnow bioassays. Dr. Ogle has specific experience in breeding these fish.

d) Dr. Thomas' laboratory at University of Texas is one of the most experienced laboratories in the country for assay of yolk and estradiol in small blood samples.

b. Schedule Milestones: see Tasks (section 2 a.). The project will be conducted and completed by the end of 1998.

c. Third Party Impacts: None

Table 2. Budget Costs

Task	Direct Labor Hours	Direct Salary & Benefits	Overhead Labor (General, Admin, & Fee)	Service Contract	Miscellaneous and Other Direct	Total cost
1. Project Management & Coordination	120	$7,281	$7,477			
Consultant: Dr. Peter Thomas[1]				$3,500		
travel, communications, misc supplies					$750	$19,008
2. Sampling	120	$3,118	$3,202			
Consultant: McClaren-Hart, Inc.[2]				$1,650		$7,970
3. Laboratory assays & analysis	98	$2,546	$2,615			
fathead minnow bioassays (30@$756)[3]			$1,914	$22,680		
estradiol & vitellogenin assays (180@$91.67)[4]			$1,393	$16,500		
travel, communications, misc supplies					$750	$48,398
4. Reporting	180	$10,922	$11,215			
travel, communications, misc supplies					$250	$22,387
Total Cost		$23,868	$27,815	$44,330	$1,750	$97,763

V. APPLICANT QUALIFICATIONS

A. ROBERT B. SPIES, Ph.D., proposed Principal Investigator

Expertise: Effects of contaminants on fishes and coastal/estuarine benthic communities; Fate and effects of organic contaminants; Biogeochemical and tracer studies; Special expertise with biomarkers and fish reproduction

Education: Ph.D.,1971, University of Southern California, Los Angeles, CA; M.S., 1969, University of Pacific, Dillon Beach, CA; B.S., 1965, St. Mary's College, Moraga , CA.

Professional Affiliations/Honors: American Association for the Advancement of Science, American Chemical Society, American Society of Limnology and Oceanography, San Francisco Bay and Estuarine Association. Past Editor, *Marine Environmental Research;* Editorial Boards of *Marine Environmental Research & Aquatic Toxicology;* American Men and Women of Science, 1977; Who's Who in California, 1982; Commendation Letter from US Attorney General, 1992.

Employment 1989-Present, President, Applied Marine Sciences, Inc., Livermore, CA; 1996-Present, Member, Board of Directors, Alaska Sealife Center, Seward, AK; 1993-Present, Member, Board of Directors, Romberg Tiburon Centers, Tiburon, CA; 1973-1993, Marine Scientist, Lawrence Livermore National Laboratories, Livermore, CA, 1970-1973, Senior Research Officer, Marine Studies Group, Ministry for Conservation, Melbourne, Australia; 1990-present: Chief Scientist, *Exxon Valdez* Oil Spill Trustee Council.

Representative publications (40+ publications)

Spies, R.B., J.S. Felton and L.J. Dillard. 1982. Hepatic mixed-function oxidases in California flatfish are increased in contaminated environments and by oil and PCB ingestion. *Mar. Biol.* 70:117–127.

Spies, R.B., B. Andresen and D.W. Rice, Jr. 1987. Benzthiazoles in estuarine sediments as indicators of street runoff. *Nature* 327:697–699.

Spies, R.B., D.W. Rice, Jr. and J.W. Felton. 1988. The effects of organic contaminants on reproduction of starry flounder, *Platichthys stellatus* (Pallas) in San Francisco Bay. Part I. Hepatic contamination and mixed-function oxidase(MFO) activity during the reproductive season. *Marine Biology* 98:181–189.

Spies, R.B. and D.W. Rice, Jr. 1988. The effects of organic contaminants on reproduction of starry flounder, *Platichthys stellatus* (Pallas) in San Francisco Bay. Part II. Reproductive success of fish captured in San Francisco Bay and spawned in the laboratory. *Marine Biology* 98:191–202.

Spies, R.B., J.J. Stegeman, D.W. Rice, Jr., B. Woodin, P. Thomas, J.E. Hose, J. Cross and M. Prieto. 1990. Sublethal responses of *Platichthys stellatus* to organic contamination in San Francisco Bay with emphasis on reproduction, pp. 87–122, in *Biological Markers of Environmental Contamination.* Lewis Publishers, Chelsea, Michigan .

Spies, R.B., J.J. Stegeman, D.E. Hinton, B. Woodin, M. Okihiro, R. Smolowitz and D. Shea.1996 Biomarkers of hydrocarbon exposure and sublethal effects in embiotocid fishes from a natural petroleum seep in the Santa Barbara Channel. *Aquatic Toxicol.* 34: 195-219.

Spies, R.B., P. Thomas, and M. Matsui. 1996. Effects of DDT and PCB on reproductive endocrinology of *Paralabrax clathratus* in southern California. Mar. Environ. Res. 42, 175-176. (abstract)

B. PETER THOMAS, Ph.D., Scientist

Education: Ph.D., Physiology, University of Leicester, England (1977); B.Sc., University of Hull, England (1970)

Honors and Professional Societies: British Fisheries Society; Society for Endocrinology; Society of Environmental Toxicology and Chemistry; Society for the Study of Reproduction

Employment: Professor, Department of Zoology, Acting Chairman, Department of Marine Science, The University of Texas at Austin, 1993-to present; Professor, Department of Marine Science, The University of Texas at Austin, 1990-present; Adjunct Professor, Texas A&M University, College Station, Texas, 1987-1990.

Selected Publications (104 total):

Khan, I. and P. Thomas. 1996. Disruption of neuroendocrine function in Atlantic croaker exposed to Aroclor 1254. Mar. Environ. Res 42:145-149.

Ghosh, P. and P. Thomas. 1995. Binding of metals to red drum vitellogenin and incorporation into oocytes. Mar. Environ. Res 39:165-168.

Ghosh, S. and P. Thomas. 1995. Antagonistic effects of xenobiotics on steroid-induced final maturation of Atlantic croaker oocytes *in vitro*. Mar. Environ. Res 39:159-163.

Thomas, P. 1993. Effects of cadmium on gonadotropin secretion from Atlantic croaker pituitaries incubated *in vitro*. Mar. Environ. Res. 35:141-145.

Thomas, P. and J. Smith. 1993. Binding of xenobiotics to the estrogen receptor of spotted seatrout A screening assay for potential estrogenic effects. Mar. Environ. Res. 35:147-151.

Thomas, P. 1988. Reproductive endocrine function in female Atlantic croaker exposed to xenobiotics. Mar. Environ. Res. 24:179-183.

C. RICHARD SCOTT OGLE, Ph.D.

Expertise: For over ten years, Dr. Scott Ogle has been directing and/or participating in research in the areas of aquatic ecotoxicology and environmental chemistry. A major area of Dr. Ogle's past research efforts has focused on factors affecting toxicity and bioaccumulation of selenium in aquatic systems, and have established him as an expert in this field. Current research activities include evaluation of the fate and

effects of petroleum and petroleum products in the aquatic environment and the investigation of contaminants and toxicity in non-point source and stormwater runoff. Dr. Ogle has directed and participated in numerous projects encompassing all of the standardized EPA and ASTM test procedures as well as projects involving research and development of new testing procedures.

Education: Ph.D. Ecology (Aquatic Ecotoxicology), 1996 , University of California, Davis, CA; M.S. Water Science (Water Pollution Biology), 1988, University of California, Davis, CA; B.S. Fisheries Biology (Water Quality), 1984, Humboldt State University, Arcata, CA

Professional Affiliations/Honors: Society of Environmental Toxicology and Chemistry (SETAC), 1989-1990 SETAC Pre-Doctoral Fellow; Northern California Regional Chapter of SETAC (NorCal SETAC), Meeting Chair for the First, Second and Third Annual NorCal SETAC Conferences, NorCal SETAC Vice-President (1990-1993), Secretary (1993-1994); Ecological Society of America; American Fisheries Society; American Association for the Advancement of Science

Employment: 1994-Present, Principal & Lab Director, Pacific Eco-Risk Labs, Martinez, CA;1991-1994, Senior Scientist, S.R. Hansen & Associates, Concord, CA; 1991, Teaching Assistant (Fish Physiology), University of California, Davis; 1986-1991, Research Assistant, University of California, Davis; 1985, Biological Aide, US Fish & Wildlife Service, Dixon, CA.

Representative Publications (15+ peer-reviewed publications/50+ technical reports):

Ogle RS, Cotsifas JS (in preparation) The role of ammonia in the toxicity of estuarine/marine sediments.

Ogle RS, Cotsifas JS (in preparation) The comparative toxicity of oil and oil products (gasolineand fuel oil) to crustaceans.

Ogle RS, Knight AW (in review) Selenium in aquatic ecosystems. 3. The roles of waterborne uptake and foodborne uptake in the bioaccumulation of selenate and selenite by fathead minnows and bluegill.

Ogle, R.S and A.W. Knight. 1996. Selenium in aquatic ecosystems. 1. Effects of sulfate on selenate uptake and toxicity in *Daphnia magna*. Archives of Environmental Contamination and Toxicology 30(2):274-279.

Saiki, M.K. and R.S. Ogle. 1995. Effects of agricultural drainwater on mosquitofish reproduction from contaminated and control field sites. Transactions American Fisheries Society 124:578-587.

Ogle, R.S. and A.W. Knight. 1989. The effects of elevated dietary selenium on growth and reproduction of the fathead minnow (*Pimephales promelas*). Archives of Environmental Contamination and Toxicology 18(6):795-805.

Ogle, R.S., K.J. Maier, P. Kiffney, M.J. Williams, A. Brasher, L.A. Melton, and A.W. Knight. 1988. Bioaccumulation of selenium in aquatic ecosystems. Lake and Reservoir Management 4(2):165-173.

Item 8

NONDISCRIMINATION COMPLIANCE STATEMENT

COMPANY NAME

Applied Marine Sciences, Inc.

The company named above (hereinafter referred to as "prospective contractor") hereby certifies, unless specifically exempted, compliance with Government Code Section 12990 (a-f) and California Code of Regulations, Title 2, Division 4, Chapter 5 in matters relating to reporting requirements and the development, implementation and maintenance of a Nondiscrimination Program. Prospective contractor agrees not to unlawfully discriminate, harass or allow harassment against any employee or applicant for employment because of sex, race, color, ancestry, religious creed, national origin, disability (including HIV and AIDS), medical condition (cancer), age, marital status, denial of family and medical care leave and denial of pregnancy disability leave.

CERTIFICATION

I, the official named below, hereby swear that I am duly authorized to legally bind the prospective contractor to the above described certification. I am fully aware that this certification, executed on the date and in the county below, is made under penalty of perjury under the laws of the State of California.

Robert B. Spies, President
OFFICIAL'S NAME

7/22/97
DATE EXECUTED

EXECUTED IN THE COUNTY OF
Alameda

PROSPECTIVE CONTRACTOR'S SIGNATURE

President
PROSPECTIVE CONTRACTOR'S TITLE

Applied Marine Sciences, Inc.
PROSPECTIVE CONTRACTOR'S LEGAL BUSINESS NAME

I-000747

Item 12

Agreement No. _____

Exhibit _____

STANDARD CLAUSES --
SMALL BUSINESS PREFERENCE AND CONTRACTOR IDENTIFICATION NUMBER

NOTICE TO ALL BIDDERS:

Section 14835, et. seq. of the California Government Code requires that a five percent preference be given to bidders who qualify as a small business. The rules and regulations of this law, including the definition of a small business for the delivery of service, are contained in Title 2, California Code of Regulations, Section 1896, et. seq. A copy of the regulations is available upon request. Questions regarding the preference approval process should be directed to the Office of Small and Minority Business at (916) 322-5060. To claim the small business preference, you must submit a copy of your certification approval letter with your bid.

Are you claiming preference as a small business?

__XX__ Yes* _____ No

*Attach a copy of your certification approval letter.

Applied Marine Sciences has applied for the small business status and is awaiting a certification approval letter. We are confident that we qualify as a small business.

I-000748