

Assessment of Hormonally Active Chemicals in the Central Valley Watershed: Monitoring, Activity Measurement, and Quantification of Adverse Effects.

Project Information

1. Proposal Title:

Assessment of Hormonally Active Chemicals in the Central Valley Watershed: Monitoring, Activity Measurement, and Quantification of Adverse Effects.

2. Proposal applicants:

Donald Michael Fry, University of California, Davis
Michael Denison, University of California, Davis
Birgit Puschner, University of California, Davis
Inge Werner, University of California, Davis
Michael Johnson, University of California, Davis

3. Corresponding Contact Person:

Ahmad Hakim-Elahi
University of California
Office of the Vice Chancellor for Research Division of Sponsored Programs Everson Hall 1
Shields Avenue University of California Davis, CA 95616
530 752-2075
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4. Project Keywords:

**Ag/Urban Runoff
Contaminants
Water Pollution, Non-point Source**

5. Type of project:

Research

6. Does the project involve land acquisition, either in fee or through a conservation easement?

No

7. Topic Area:

Ecosystem Water and Sediment Quality

8. Type of applicant:

University

9. Location - GIS coordinates:

Latitude: 38.543

Longitude: -121.756

Datum:

Describe project location using information such as water bodies, river miles, road intersections, landmarks, and size in acres.

Water samples will be collected from many sites in the Sacramento and San Joaquin watersheds, and processed at UC Davis.

10. Location - Ecozone:

3.2 Red Bluff Diversion Dam to Chico Landing, 3.3 Chico Landing to Colusa, 3.4 Colusa to Verona, 3.5 Verona to Sacramento, 6.1 Stony Creek, 6.2 Elder Creek, 6.3 Thomas Creek, 6.4 Colusa Basin, 7.7 Butte Sink, 8.1 Feather River, 8.4 Sutter Bypass, 9.1 American Basin, 9.2 Lower American River, 10.1 Cache Creek, 10.2 Putah Creek, 10.3 Solano, 10.4 Willow Slough, 12.1 Vernalis to Merced River, 12.2 Merced River to Mendota Pool, 12.3 Mendota Pool to Gravelly Ford, 13.1 Stanislaus River, 13.2 Tuolumne River, 13.3 Merced River, West San Joaquin Basin, 1.1 North Delta, 1.2 East Delta, 1.3 South Delta, 1.4 Central and West Delta, 11.1 Cosumnes River, 11.2 Mokelumne River, 11.3 Calaveras River

11. Location - County:

Amador, Butte, Colusa, Contra Costa, El Dorado, Fresno, Glenn, Madera, Merced, Placer, Sacramento, San Joaquin, Solano, Stanislaus, Sutter, Tehama, Yolo, Yuba

12. Location - City:

Does your project fall within a city jurisdiction?

No

13. Location - Tribal Lands:

Does your project fall on or adjacent to tribal lands?

No

14. Location - Congressional District:

3rd.

15. Location:

California State Senate District Number: 4

California Assembly District Number: 8

16. **How many years of funding are you requesting?**

3

17. **Requested Funds:**

a) Are your overhead rates different depending on whether funds are state or federal?

Yes

If yes, list the different overhead rates and total requested funds:

State Overhead Rate: 10%

Total State Funds: 1,466,992.30

Federal Overhead Rate: 48.5%

Total Federal Funds: 1,838,343.10

b) Do you have cost share partners already identified?

No

c) Do you have potential cost share partners?

No

d) Are you specifically seeking non-federal cost share funds through this solicitation?

No

If the total non-federal cost share funds requested above does not match the total state funds requested in 17a, please explain the difference:

18. **Is this proposal for next-phase funding of an ongoing project funded by CALFED?**

No

Have you previously received funding from CALFED for other projects not listed above?

Yes

If yes, identify project number(s), title(s) and CALFED program.

B-81609	Monitoring and Mitigating Offsite Movement of Dormant Spray Pesticides from California Orchards	Ecosystem Restoration
99-N08	Pesticide Effects on Fish and their Food Resources in the Sacramento-San Joaquin Delta	Ecosystem Restoration
99-N07	Chronic Toxicity of Environmental Contaminants in Sacramento Splittail	Ecosystem Restoration

19. **Is this proposal for next-phase funding of an ongoing project funded by CVPIA?**

No

Have you previously received funding from CVPIA for other projects not listed above?

No

20. **Is this proposal for next-phase funding of an ongoing project funded by an entity other than CALFED or CVPIA?**

No

Please list suggested reviewers for your proposal. (optional)

Steven Goodbred USGS, Sacramento goodbred@usgs.org

Jay Davis SFEI, Richmond CA jay@sfei.org

21. **Comments:**

Environmental Compliance Checklist

Assessment of Hormonally Active Chemicals in the Central Valley Watershed: Monitoring, Activity Measurement, and Quantification of Adverse Effects.

1. CEQA or NEPA Compliance

- a) Will this project require compliance with CEQA?

No

- b) Will this project require compliance with NEPA?

No

- c) If neither CEQA or NEPA compliance is required, please explain why compliance is not required for the actions in this proposal.

This is a project which will not have a significant effect on the environment. Water sampling will be the only field work in this study.

2. If the project will require CEQA and/or NEPA compliance, identify the lead agency(ies). *If not applicable, put "None".*

CEQA Lead Agency:

NEPA Lead Agency (or co-lead:)

NEPA Co-Lead Agency (if applicable):

3. Please check which type of CEQA/NEPA documentation is anticipated.

CEQA

Categorical Exemption

-Negative Declaration or Mitigated Negative Declaration

-EIR

-none

NEPA

Categorical Exclusion

-Environmental Assessment/FONSI

-EIS

-none

If you anticipate relying on either the Categorical Exemption or Categorical Exclusion for this project, please specifically identify the exemption and/or exclusion that you believe covers this project.

projects which the Secretary of the Resources Agency has determined do not have a significant effect on the environment.

4. CEQA/NEPA Process

- a) Is the CEQA/NEPA process complete?

Not Applicable

b) If the CEQA/NEPA document has been completed, please list document name(s):

5. **Environmental Permitting and Approvals** (*If a permit is not required, leave both Required? and Obtained? check boxes blank.*)

LOCAL PERMITS AND APPROVALS

Conditional use permit

Variance

Subdivision Map Act

Grading Permit

General Plan Amendment

Specific Plan Approval

Rezone

Williamson Act Contract Cancellation

Other

STATE PERMITS AND APPROVALS

Scientific Collecting Permit

CESA Compliance: 2081

CESA Compliance: NCCP

1601/03

CWA 401 certification

Coastal Development Permit

Reclamation Board Approval

Notification of DPC or BCDC

Other

FEDERAL PERMITS AND APPROVALS

ESA Compliance Section 7 Consultation

ESA Compliance Section 10 Permit

Rivers and Harbors Act

CWA 404

Other

PERMISSION TO ACCESS PROPERTY

Permission to access city, county or other local agency land.

Agency Name:

Permission to access state land.

Agency Name:

Permission to access federal land.

Agency Name:

Permission to access private land.

Landowner Name:

6. Comments.

Water sampling will be conducted generally from public rights of way, including roads and bridges over waterways.

Land Use Checklist

Assessment of Hormonally Active Chemicals in the Central Valley Watershed: Monitoring, Activity Measurement, and Quantification of Adverse Effects.

1. **Does the project involve land acquisition, either in fee or through a conservation easement?**

No

2. **Will the applicant require access across public or private property that the applicant does not own to accomplish the activities in the proposal?**

No

3. **Do the actions in the proposal involve physical changes in the land use?**

No

If you answered no to #3, explain what type of actions are involved in the proposal (i.e., research only, planning only).

Water sampling for research only.

4. **Comments.**

Conflict of Interest Checklist

Assessment of Hormonally Active Chemicals in the Central Valley Watershed: Monitoring, Activity Measurement, and Quantification of Adverse Effects.

Please list below the full names and organizations of all individuals in the following categories:

- Applicants listed in the proposal who wrote the proposal, will be performing the tasks listed in the proposal or who will benefit financially if the proposal is funded.
- Subcontractors listed in the proposal who will perform some tasks listed in the proposal and will benefit financially if the proposal is funded.
- Individuals not listed in the proposal who helped with proposal development, for example by reviewing drafts, or by providing critical suggestions or ideas contained within the proposal.

The information provided on this form will be used to select appropriate and unbiased reviewers for your proposal.

Applicant(s):

Donald Michael Fry, University of California, Davis
Michael Denison, University of California, Davis
Birgit Puschner, University of California, Davis
Inge Werner, University of California, Davis
Michael Johnson, University of California, Davis

Subcontractor(s):

Are specific subcontractors identified in this proposal? No

Helped with proposal development:

Are there persons who helped with proposal development?

No

Comments:

Budget Summary

Assessment of Hormonally Active Chemicals in the Central Valley Watershed: Monitoring, Activity Measurement, and Quantification of Adverse Effects.

Please provide a detailed budget for each year of requested funds, indicating on the form whether the indirect costs are based on the Federal overhead rate, State overhead rate, or are independent of fund source.

Federal Funds

Year 1												
Task No.	Task Description	Direct Labor Hours	Salary (per year)	Benefits (per year)	Travel	Supplies & Expendables	Services or Consultants	Equipment	Other Direct Costs	Total Direct Costs	Indirect Costs	Total Cost
1	water sampling	1692	37,678.68	7,134.38	5880	4000	1500	2500		58693.06	27253.63	85946.69
2	hormone activity	1020	14580	3207.60	0	5000	0	0		22787.6	11051.99	33839.59
3	chemistry	1020	14850	3267.00	0	15000		403490		436607.0	16061.75	452668.75
4	fish effects	4258	82219.8	15249.52	0	21500	0	0		118969.32	57700.12	176669.44
5	program management	768	26400.68	4487.78	1500	1450	0	0		33838.46	16411.65	50250.11
		8758	175729.16	33346.28	7380.00	46950.00	1500.00	405990.00	0.00	670895.44	128479.14	799374.58

Year 2												
Task No.	Task Description	Direct Labor Hours	Salary (per year)	Benefits (per year)	Travel	Supplies & Expendables	Services or Consultants	Equipment	Other Direct Costs	Total Direct Costs	Indirect Costs	Total Cost
1	water sampling	1176	39562.61	7491.09	6000	4000	1500	0	0	58553.7	28398.55	86952.25
2	hormone activity	1020	15309	3367.98	0	5000	0	0	0	23676.98	11483.34	35160.32
3	chemistry	1020	15592.5	3430.35	0	15000	0	0	0	34022.85	16501.08	50523.93
4	fish effects	5482	133020	25170.66	0	28000	0	0	0	186190.66	90302.47	276493.13
5	program management	768	27720.61	4712.1	4000	1900	0	0	0	38332.71	18591.36	56924.07
		9466	231204.72	44172.18	10000.00	53900.00	1500.00	0.00	0.00	340776.90	165276.80	506053.70

Year 3												
Task No.	Task Description	Direct Labor Hours	Salary (per year)	Benefits (per year)	Travel	Supplies & Expendables	Services or Consultants	Equipment	Other Direct Costs	Total Direct Costs	Indirect Costs	Total Cost
1	water sampling	1176	41540.74	7865.65	6000	4000	1500	0	0	60906.39	29539.60	90445.99
2	hormone activity	1020	15309	3367.98	0	5000	0	0	0	23676.98	11483.34	35160.32
3	chemistry	1020	16372.13	3601.87	0	15000	0	0	0	34974.0	16962.39	51936.39
4	fish effects	5482	139666	26427.25	0	29000	0	0	0	195093.25	94620.23	289713.48
5	program management	876	32747.3	5567.27	4000	1900	0	0	0	44214.57	21444.07	65658.64
		9574	245635.17	46830.02	10000.00	54900.00	1500.00	0.00	0.00	358865.19	174049.63	532914.82

Grand Total=1838343.10

Comments.

Budget Justification

Assessment of Hormonally Active Chemicals in the Central Valley Watershed: Monitoring, Activity Measurement, and Quantification of Adverse Effects.

Direct Labor Hours. Provide estimated hours proposed for each individual.

Direct Labor Hours: Much time will be provided gratis to this project by faculty at the University. The effort by faculty and researchers is presented below, but not included in the budget forms for those Co-Principal Investigators not taking salary for this project. Year 1: Task 1: Water sampling and fractionation Michael Fry: 672 hours. All sampling decisions, locations, planning. Supervision of sampling, extractions, fractionations, archiving. Staff Research Associate: 1020 hours. All sample collections, data entry, solvent extractions, storage. Task 2: Hormone activity measurements Michael Dennison: 90 hours. No cost to project. Supervision of hormone assays, data supervision Staff Research Associate: 1020 hours. Hormone assay measurements, cell cultures, data entry. Task 3: Analytical Chemistry Birgit Puschner: 90 hours. No cost to project. Supervision of analytical chemistry. Data management. Elizabeth Tor: 90 hours. No cost to project. Supervision of analytical instruments and laboratory work. Data analysis. Post Graduate Resaercher: 1020 hours. Technical operation of analytical instruments, data entry, archiving of samples and data. Task 4: Laboratory fish studies, biomarker and histopathology Inge Werner: 50 hours. No cost to project. Supervision of biomarkers laboratory, development of vitellogenin and choriogenin assays. Michael Johnson: 50 hours. No cost to project. Supervision of Post-docoral researcher. Linda Hall, Postdoctoral researcher. 2040 hours. Development of TAQMAN assays, assays for fish protein biomarkers. Swee Teh: 202 hours. Supervision of aquaculture laboratory, supervision of fish histology/pathology preparation. Reading and interpretation of histology slides. Staff Research Associate IV: 1008 hours. Daily operation of aquaculture facility. Dosing and maintenance of fish. Lab Assistant 4: 504 hours. Daily operation of aquaculture facility. Histology preparations. Student Assistants: 504 hours. Care of fish and maintenance of facility. Task 5: Project Management: Michael Fry: 672 hours: Project administration. Database management. Report preparation and presentation. Consultation with Agencies, Agricultural Commissioners offices, Municipalities. GPS database and trip log data management. Inge Werner: 96 hours: Task 4 project administration. Report preparation and presentation. Swee Teh: 50 hours. No cost to project. Task 4 project administration. Report preparation and presentation. Year 2: Task 1: Water sampling and fractionation Michael Fry: 672 hours. All sampling decisions, locations, planning. Supervision of sampling, extractions, fractionations, archiving. Staff Research Associate: 1020 hours. All sample collections, data entry, solvent extractions, storage. Task 2: Hormone activity measurements Michael Dennison: 90 hours. No cost to project. Supervision of hormone assays, data supervision Staff Research Associate: 1020 hours. Hormone assay measurements, cell cultures, data entry. Task 3: Analytical Chemistry Birgit Puschner: 90 hours. No cost to project. Supervision of analytical chemistry. Data management. Elizabeth Tor: 90 hours. No cost to project. Supervision of analytical instruments and laboratory work. Data analysis. Post Graduate Resaercher: 1020 hours. Technical operation of analytical instruments, data entry, archiving of samples and data. Task 4: Laboratory fish studies, biomarker and histopathology Inge Werner: 408 hours. Supervision of biomarkers laboratory, development and conduction of vitellogenin and choriogenin assays. Laboratory Assistant: 816 hours. Laboratory assays, maintenance of lab. Michael Johnson: 50 hours. No cost to project. Supervision of Post-docoral researcher. Linda Hall, Postdoctoral researcher. 2040 hours. Development of TAQMAN assays, assays for fish protein biomarkers. Swee Teh: 504 hours. Supervision of aquaculture laboratory, supervision of fish histology/pathology preparation. Reading and interpretation of histology slides. Staff Research Associate IV: 1008 hours. Daily operation of aquaculture facility. Dosing and maintenance of fish. Lab Assistant 4: 1008 hours. Daily operation of aquaculture facility. Histology preparations. Dosing of fish. Student Assistants: 504 hours. Care of fish and maintenance of facility.

Task 5: Project Management: Michael Fry: 672 hours: Project administration. Database management. Report preparation and presentation. Consultation with Agencies, Agricultural Commissioners offices, Municipalities. GPS database and trip log data management. Inge Werner: 96 hours: Task 4 project administration. Report preparation and presentation. Swee Teh: 50 hours. No cost to project. Task 4 project administration. Report preparation and presentation. Year 3: Task 1: Water sampling and fractionation Michael Fry: 672 hours. All sampling decisions, locations, planning. Supervision of sampling, extractions, fractionations, archiving. Staff Research Associate: 1020 hours. All sample collections, data entry, solvent extractions, storage. Task 2: Hormone activity measurements Michael Dennison: 90 hours. No cost to project. Supervision of hormone assays, data supervision Staff Research Associate: 1020 hours. Hormone assay measurements, cell cultures, data entry. Task 3: Analytical Chemistry Birgit Puschner: 90 hours. No cost to project. Supervision of analytical chemistry. Data management. Elizabeth Tor: 90 hours. No cost to project. Supervision of analytical instruments and laboratory work. Data analysis. Post Graduate Resaercher: 1020 hours. Technical operation of analytical instruments, data entry, archiving of samples and data. Task 4: Laboratory fish studies, biomarker and histopathology Inge Werner: 408 hours. Supervision of biomarkers laboratory, development and conduction of vitellogenin and choriogenin assays. Laboratory Assistant: 816 hours. Laboratory assays, maintenance of lab. Michael Johnson: 50 hours. No cost to project. Supervision of Post-docoral researcher. Linda Hall, Postdoctoral researcher. 2040 hours. Development of TAQMAN assays, assays for fish protein biomarkers. Swee Teh: 504 hours. Supervision of aquaculture laboratory, supervision of fish histology/pathology preparation. Reading and interpretation of histology slides. Staff Research Associate IV: 1008 hours. Daily operation of aquaculture facility. Dosing and maintenance of fish. Lab Assistant 4: 1008 hours. Daily operation of aquaculture facility. Histology preparations. Dosing of fish. Student Assistants: 504 hours. Care of fish and maintenance of facility. Task 5: Project Management: Michael Fry: 672 hours: Project administration. Database management. Report preparation and presentation. Consultation with Agencies, Agricultural Commissioners offices, Municipalities. GPS database and trip log data management. Inge Werner: 202 hours: Task 4 project administration. Report preparation and presentation. Swee Teh: 50 hours. No cost to project. Task 4 project administration. Report preparation and presentation.

Salary. Provide estimated rate of compensation proposed for each individual.

Salary: Salaries are based on University of California compensation tables. All figures are Monthly Salary for full time (170 hours per month). Salary figures for Years 2 and 3 are increased by 5% each year. Michael Fry: Research Physiologist: \$5833 Staff Research Associate: \$2430 Michael Dennison: Professor: \$6350. No cost to project Staff Research Associate: \$2430 Birgit Puschner: Assistant Professor, Toxicologist: \$5340. No cost to project Elizabeth Tor: Staff Resaerch Associate IV: \$5320. No cost to project Post Graduate Resaercher: \$2475. Inge Werner: Assistant Researcher: \$5500 Laboratory Assistant: \$2524 Michael Johnson: Professor: \$6350. No cost to project. Linda Hall, Postdoctoral researcher. \$2300 Swee Teh: Research Toxicologist/Pathologist: \$5833 Staff Research Associate IV: \$5420 Lab Assistant 4: \$3333 Student Assistants: \$1600

Benefits. Provide the overall benefit rate applicable to each category of employee proposed in the project.

Benefits: Faculty benefit rate: 17% Staff Benefit rate: 23% Student benefit rate: 0.05%

Travel. Provide purpose and estimate costs for all non-local travel.

Travel: Year 1: Task 1: \$5880, includes University motor pool charges of \$43.35/day plus \$0.07/mile for 50 sampling trips of approximately 200 miles each. Overnight and per diem charges are included for 10 trips. Task 5: \$1500 for travel to Agencies, Counties, and Presentations. Year 2: Task 1: \$6000

for sample collection, based on same formula as Year 1 Task 5: \$4000 for travel to scientific meetings and presentations for 4 researchers Year 3: Task 1: \$6000 for sample collection, based on same formula as Year 1 Task 5: \$4000 for travel to scientific meetings and presentations for 4 researchers

Supplies & Expendables. Indicate separately the amounts proposed for office, laboratory, computing, and field supplies.

Year 1: Task 1: \$4000, for solid phase extraction equipment, pump, solvents, and photographic documentation. Task 2: \$5000, for culture supplies, reagents for hormone assays. Task 3: \$15000 for GC and HPLC columns, chemicals, solvents, standards. Task4: \$21500 for aquarium supplies, filters, live fish, histology supplies, photographic supplies. Task5: \$1450 for office supplies, telephone charges, publication costs. Year 2: Task 1: \$4000, for solid phase extraction equipment, pump, solvents, and photographic documentation. Task 2: \$5000, for culture supplies, reagents for hormone assays. Task 3: \$15000 for GC and HPLC columns, chemicals, solvents, standards. Task4: \$28000 for aquarium supplies, filters, live fish, histology supplies, photographic supplies. Task5: \$1900 for office supplies, telephone charges, publication costs. Year 3: Task 1: \$4000, for solid phase extraction equipment, pump, solvents, and photographic documentation. Task 2: \$5000, for culture supplies, reagents for hormone assays. Task 3: \$15000 for GC and HPLC columns, chemicals, solvents, standards. Task4: \$29000 for aquarium supplies, filters, live fish, histology supplies, photographic supplies. Task5: \$1900 for office supplies, telephone charges, publication costs.

Services or Consultants. Identify the specific tasks for which these services would be used. Estimate amount of time required and the hourly or daily rate.

Year 1: Task 1: \$1500 for chemical disposal services, Environmental Health and Safety Year 2: Task 1: \$1500 for chemical disposal services, Environmental Health and Safety Year 3: Task 1: \$1500 for chemical disposal services, Environmental Health and Safety

Equipment. Identify non-expendable personal property having a useful life of more than one (1) year and an acquisition cost of more than \$5,000 per unit. If fabrication of equipment is proposed, list parts and materials required for each, and show costs separately from the other items.

Year 1: Task 1: \$2500 Laptop computer with GPS software and antenna for sample collection documentation and mapping. Task 3: \$403490 Analytical chemistry instrumentation to augment current laboratory equipment used full time by laboratory. Diagnostic samples are run 24 hr/day currently, and additional equipment is necessary for this project. Current equipment will also be used, as this equipment augments, but does not replace current gas and liquid chromatographs and mass spectrometers. 1. Tandem Mass Spectrometer ThermoFinnigan Model TSQ Quantum \$330,000 Electrospray + APCI probe \$11,000 Surveyor Kit \$525 Liquid chromatograph (Surveyor MS Pump w/degasser) \$27795 Autosampler (Surveyor MS) \$19000 PDA Detector (Surveyor, optional) \$14700 IBM Pentium Pro computer, CD-ROM drive Extended warranty Second year service contract Three course package Sub-Total: \$403,020 Less Promotional Discount (22.1%) \$89,020 Total: \$314,000 (Quote on 5/3/01) New technology, currently not in the lab., needed for detection of steroids, herbicides, polar pesticides at low detection limits 2. HPLC/DAD/Fluorescence Detectors Agilent Model 1100 HPLC \$67,490 (quote 4/2/01) Second year service contract 3. Post Column Derivatization System: \$ 14,000 Currently used on the old Perkin Elmer Series 4 HPLC for derivations of certain chemicals like carbamate insecticides to enhance their ability to be detected by HPLC. Current system is very old and may need to be replaced. 4. Nitrogen Evaporator: Zymark Corporation \$8,000 Years 2 and 3: No equipment.

Project Management. Describe the specific costs associated with insuring accomplishment of a specific project, such as inspection of work in progress, validation of costs, report preparation, giving presentations, response to project specific questions and necessary costs directly associated with specific project oversight.

Fry will spend 1/3 full time on project administration, database management, consultation with Agricultural commissioners, CA DPR, GPS mapping, and report preparation. All data from Tasks 1, 2, 3, and 4 will be correlated and archived. Outreach and project milestones will be monitored by attendance of Scientific meetings and presentations to Agencies. Preparation for publication will be a primary activity for Fry and Werner in Years 2 and 3.

Other Direct Costs. Provide any other direct costs not already covered.

None

Indirect Costs. Explain what is encompassed in the overhead rate (indirect costs). Overhead should include costs associated with general office requirements such as rent, phones, furniture, general office staff, etc., generally distributed by a predetermined percentage (or surcharge) of specific costs.

Federal Indirect Cost Rate is 48.5% of modified direct costs. No indirect costs are taken for equipment. The University supplies office and laboratory space, water, plumbing, electricity, building maintenance and janitorial staff. Each Department is reimbursed for cost of office staff participation in research projects, including publications, budget management, and purchasing. State Indirect Cost has been negotiated at 10% of modified direct costs. State general funds are used to support the University, and the lower negotiated indirect costs reflect direct State support of the University.

Executive Summary

Assessment of Hormonally Active Chemicals in the Central Valley Watershed: Monitoring, Activity Measurement, and Quantification of Adverse Effects.

Executive Summary: Assessment of Hormonally Active Chemicals in the Central Valley Watershed: Monitoring, Activity Measurement, and Quantification of Adverse Effects. DM Fry, MS Denison, ML Johnson, I Werner, and SJ Teh. Hormonally active chemicals, also called endocrine disruptors, have been detected in water samples from the Central Valley watershed (Johnson et al. 1998). The magnitude, watershed geographical distribution, and identity of the active compounds are unknown. Whether these chemicals are present at concentrations sufficient to cause adverse effects in fish, wildlife, and humans is also unknown. CALFED has included specific objectives with respect to pesticides and other pollutants with hormonal activity in the Multi-Region Priority 5: Environmental Water Quality, and in Goal 6 of the Draft Stage 1 Implementation Plan of the Ecosystem Restoration Program. This proposal: 1) will provide a detailed assessment of chemicals present in the Sacramento-San Joaquin watersheds which have reproductive hormonal activity; 2) will identify and quantify those chemicals; 3) will determine the adverse effects of individual chemicals on the mosquito fish (*Gambusia affinis*), an introduced fish widely distributed throughout the watershed; and 4) will provide an assessment as to the environmental hazard, if any, posed by these compounds. We plan to collect more than 1000 water samples per year, beginning with 8 Central Valley Counties and expanding to 13 Counties in Years 2 and 3. Principle agricultural drains, key river segments, and municipal outfalls will be sampled. Water samples will be screened using highly sensitive, specific estrogen and androgen bio-assays to detect and quantify hormonal activity. Water samples with significant activity will be fractionated and analyzed by the Toxicology Division of the California Animal Health and Food Safety Laboratory, using the most current analytical techniques available. The results will determine which agricultural and household chemicals with known hormonal activity are entering the watershed, and this study will most probably identify additional active compounds that have never been identified as endocrine disruptors. We will correlate chemicals in the watershed with pesticide use reports compiled by the California Department of Pesticide Regulation (CA DPR), and repeatedly sample exposed areas of the watershed to confirm and quantify the magnitude of watershed contamination by agricultural chemicals. We will also reconfirm and quantify any chemicals from municipal outfalls. The identified chemicals with the greatest potential for causing adverse effects in fish and humans will be further tested in the laboratory to determine a dose-response for reproductive impairment of adult mosquito fish, and for developmental impairment of embryo mosquito fish. The dose-response data will be compared to the water sample concentrations of the identified chemicals, to assess the potential for environmental injury. The results of this study will be published in peer-reviewed journals, as well as reported to CALFED, the US EPA and CA DPR.

Proposal

University of California, Davis

**Assessment of Hormonally Active Chemicals in the Central Valley Watershed:
Monitoring, Activity Measurement, and Quantification of Adverse Effects.**

Donald Michael Fry, University of California, Davis

Michael Denison, University of California, Davis

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Michael Johnson, University of California, Davis

Assessment of Hormonally Active Chemicals in the Central Valley Watershed:
Monitoring, Activity Measurement, and Quantification of Adverse Effects.
DM Fry, MS Denison, ML Johnson, I Werner, and SJ Teh.

A. Project description: project goals and scope of work

1. **Problem:** Hormonally active chemicals are present in the Central Valley watershed (Johnson et al. 1998). The geographical extent of the chemicals is unknown. Whether the chemicals are present at levels that pose a risk to fish or humans is unknown.

This study will determine the geographical extent and magnitude of hormonal activity throughout the Sacramento and San Joaquin watersheds, will determine which chemicals are responsible, and will determine whether the chemicals pose a risk to the environment or to humans. Hormonally active chemicals have the potential to cause significant environmental injury, and a detailed assessment of the chemicals present in the watershed should be conducted.

2. **Justification:**

Conceptual Model:

Many vertebrates and invertebrate animals rely upon steroid hormones to control the differentiation of the reproductive tracts of embryos, and to initiate and regulate reproductive cycles in adult animals. The presence of endocrine active chemicals in the watershed has been correlated with anatomical and physiological abnormalities and fish in the UK (Sumpter et al Harries et al, 1997, 1998, Jobling et al 1998,), and in Lake Mead, NV (Snyder et al. 2001a, 2001b). More than 46 agricultural, pharmaceutical, and industrial chemicals identified as hormonally active agents by the National Academy Panel on Hormonally Active Agents in the Environment (NRC, NAS 1999), although only a few compounds have, to date, been correlated with adverse effects on fish and wildlife in the field. High concentrations of DDT in the southern California coastal ecosystem were correlated with injury to fish, marine mammals, and several species and birds (Fry and Toone 1981, Fry et al. 1987), alkylphenolic detergents were identified as the cause of impaired fish reproduction in the UK (Sumpter et al.1998, Jobling et al.1998), and ethynyl estradiol, a human urinary excretory product from contraceptive use, has been identified both in the UK and in Nevada as interfering with fish reproduction. Significant levels of estrogenic compounds have been detected in agricultural drain water in San Joaquin County, CA (Johnson et al. 1998), although in this preliminary study we made no attempt to identify the chemicals present.

This proposal is the logical extension of our pilot study, and is designed not only to identify areas of the watershed with significant aqueous waterborne hormonal activity, but also to identify the chemicals responsible, determine the levels of the compounds which will cause adverse effects in a common fish species within the watershed, and correlate the adverse effects with watershed exposure.

It is known from agricultural use records maintained by the California department of pesticide regulation (DPR) that more than one million pounds of endocrine active compounds are used annually by commercial California agriculture. The magnitude of release of hormonally active

industrial compounds, especially those not listed in the US EPA toxic release inventory, is unknown, and the levels of hormonally active compounds released from municipal wastewater districts, consisting of pharmaceuticals and cosmetics and many backyard unregulated landscape chemicals, has not been monitored. This project is designed to evaluate these sources, approaching the question initially from the hormonal activity present in the water, followed by chemical identification of active compounds, and assessment of their potential for adverse environmental effects.

Our plan to screen samples based on the hormonal activity in water samples is based on two grounds: Possible adverse environmental effects are based on the sum-total of hormonal activity present in a given water sample, which may be due to a combination of chemicals; and many of the chemicals responsible for the hormonal activity have not been identified, because the hormonal screening planned by the US EPA under the requirements of the Food Quality Protection Act of 1996, has not been implemented. This study will not only identify the environmental risk, but most likely will additionally identify a number of previously unrecognized hormonally active chemicals. This may be especially true for new herbicides and insecticides recently introduced to replace older chemicals. To accomplish this project, we have assembled a team of scientists with national and international recognition in the field of endocrine disruption, chemical analysis, and assessment of the physiological effects toxicants to fish.

Hypotheses:

Hypothesis 1: Hormonally active compounds are present in the watershed at levels that can disrupt reproduction and development of fishes.

Hypothesis 1A: Water samples taken from the watershed can be analyzed for hormonal activity, and samples which test positive can be analyzed by analytical techniques to identify the active compounds.

Hypothesis 1B: Hormonal activity in a single water sample may be due to a combination of chemicals, some with agonistic and some with antagonistic activities.

Hypothesis 1C: Testing mosquito fish (*Gambusia*) with pure chemicals in the laboratory will establish the environmental levels of the chemical that will cause adverse effects.

The final comparison between the levels of compounds found in the environmental water samples and the adverse effect levels determined with *Gambusia* in the laboratory will ultimately determine whether the hormonally active chemicals are present in the environment at hazardous levels.

We anticipate that field collections of *Gambusia* and other species of fish from any adversely affected areas of the watershed will be required to confirm these laboratory results.

Adaptive Management Diagram relationships:

Three levels of adaptive management will occur in this study:

1. Water sampling locations and sampling times will be adapted to reflect positive hits, both by re-sampling hot sites, and by comparing DPR use reports and sampling downstream of high use areas of positive compounds.

2. The laboratory procedures for sample fractionation modified as needed to reflect the level of separation required for analytical analysis by LC MS and other techniques used by the CAHFS lab.
3. The selection of chemicals for Task 4, fish evaluation, will be based on consideration of the amount of each possible chemical applied or used in the watershed, the level of understanding of the activity of the chemical from other studies, the number of positive hits from Tasks 2 and 3, and consultation with CALFED, DPR, and USEPA.

3. Approach:

The work proposed here is organized into five tasks:

Task 1. Water sampling and sample fractionation.

Task 2. Hormonal activity screening, including initial screening and screening of sample fractions.

Task 3. Analytical identification of active fractions.

Task 4. Laboratory fish studies using *Gambusia*: This task includes fish culture, biomarker studies to identify physiological responses and adverse effects in adult fish, and evaluation of dose response and adverse effects in developing fish.

Task 5. Project management: This task includes adaptive management of sampling design and sampling locations; correlation of watershed exposure with DPR use reports; outreach, including presentations, publications, and information transfer to agencies; data management; and quality assurance.

Task 1: water sampling and sample fractionation.

We plan to collect approximately 100 water samples per month, beginning in Year 1 with sampling from eight counties in the Sacramento and San Joaquin drainages. The initial eight counties will be: Sutter, Colusa, Yolo, Sacramento, Solano, San Joaquin, Stanislaus, and Merced. We plan to sample major agricultural drains and their tributaries, convergence points of agricultural drains and creeks or rivers, and municipal water treatment plant outfalls. Prior to sampling we will confer with other projects conducting sampling (Kathy Kuivila, USGS, Charlie Kratzer, USGS, the Department of Water Resources, the Central Valley Regional Water Quality Control Board, Municipal water treatment facilities, and with the agricultural commissioners' office in each of the eight counties to coordinate sampling and to obtain recommendations for the best local sampling sites. In Year 2, we plan to increase the geographical scope of water sampling, to include parts of Tehama, Glenn, Butte, Yuba, Madera, Contra Costa and Fresno Counties, and possible inclusion of parts of Placer, El Dorado, and Amador Counties, depending upon DPR use reports of hormonally active chemicals. We anticipate the total number of samples to remain approximately constant between years, by altering the sample frequency for individual sites, or deletion of sites based on the results of year 1.

Water sampling will be conducted using a protocol similar to that of Snyder et al. (2001a,b), in which 5 liter water samples were pumped through solid phase extraction cartridges or disks in the field. Each sample cartridge/disk will be identified in the field with a bar-code label, with data entered into a GPS database in a laptop computer in the field. The trip-log and data entries will be archived for each sample collection trip to positively identify the time and location of each sample collection.

It is likely that this sampling and extraction protocol will lose pyrethroid insecticides, because of their tendency to bind to surfaces and matrices. Developing sampling techniques for pyrethroids is a significant study on its own, and beyond the scope of this project. We will work closely with the USGS proposed CALFED project titled "Pyrethroid Insecticides: Analysis, Occurrence, and Fate in the Sacramento and San Joaquin Rivers and Delta", if that project is funded. We have conferred with Kathy Kuivila, a participant in that proposal, and have offered to test samples of chemicals detected by that study for hormone activity, and to work with their program in coordination of sample collection and analysis.

Solid phase cartridges/disks will be extracted using the method of Snyder et al (2001, a,b) and an aliquot of each sample will be delivered to Denison's laboratory for hormonal activity analysis in Task 2. The majority of the sample will be stored frozen at -20° C. until the sample is selected for further fractionation and analytical chemistry identification of active compounds.

Samples will be selected for further analysis based on hormonal activity. The sample separation, based on the method of Snyder et al. (2001) will separate samples into polar, non-polar, and semi-polar fractions suitable for analysis by the toxicology Section of the California Animal Health and Food Safety Laboratory (CAHFS) in Task 3. Each sub-fraction will be re-analyzed for hormonal activity, prior to submission of the active fraction(s) to CAHFS.

Task 2: Hormonal Activity Screening:

Measurement of estrogenic and androgenic activity present in sample extracts will be carried out using recombinant cell bioassay systems that we have previously described (Rogers and Denison, 2000). A recombinant human ovarian carcinoma (BG-1) cell line (called BG1Luc4E₂ cells) was generated that contains a stably-transfected estrogen-responsive firefly luciferase reporter gene. These cells respond in a time, dose and chemical specific manner-dependent manner to 17β-estradiol and related xenoestrogens (including o'p'-DDT, methoxychlor, kepone, bisphenol a, nonylphenol and other chemicals) with the induction of up to a 100-fold induction of luciferase activity. In addition, this cell bioassay extremely sensitive, with a lower limit of detection of at least 0.1 pM of estradiol (Rogers and Denison 2000). In addition to estrogenic activity, we also propose to examine the androgenic activity of the same samples using a recombinant prostate cell line (LNCaP) that was transiently transfected with an androgen-responsive luciferase reporter gene. In our previous studies (Rogers and Denison, 2000) we demonstrated the feasibility of this system to detect androgens (i.e. testosterone) and cells stably transfected with this androgen-responsive reporter gene are currently being generated by an identical approach used to make the estrogen-responsive cell bioassay. The utility of cell bioassays for endocrine disrupting chemicals as screening assays have been described (reviewed in Reel et al., 1996; Gaido et al., 1997; Gray et al., 1997; Legler et al., 1999). These new recombinant cell bioassays have not only resulted in the identification of a novel endocrine disrupting chemicals, but they provide researchers with a simple, sensitive and relatively inexpensive approach for screening large number of samples, such as that described in this proposal (Pons et al., 1990; Garrison et al., 1996; Barton and Anderson, 1998; Kelce et al., 1998; Kuil et al., 1998; Cheek et al., 1999; Go et al., 1999; Legler et al., 1999).

For our experiments, water samples collected on solid phase cartridges will be extracted using procedures we and others have previously described for the isolation of hydrophobic and

hydrophilic substances (Eide, 1996; Murk et al., 1996, 1997; Denison et al., 1996; Ostby et al., 1997; Feron et al. 1998, Snyder et al. 2001). Initial screening analysis for estrogenic and androgenic activity will involve exposing cells grown in 96-well microplates to an aliquot (2 μ l) of each sample extract for 24 hours followed by measurement of luciferase activity and normalization to protein concentration as we have previously described in detail (Garrison et al., 1996; Rogers and Denison, 2000). Luciferase activity of all samples will be compared to activity obtained with both negative controls (solvent alone) and positive controls (estrogen or testosterone) and those samples exhibiting positive activity will be analyzed further.

In order to determine the relative estrogenic/androgenic potency of a given sample extract, cells will be exposed to 5-8 serial dilutions of the sample extract and luciferase activity determined. Concurrent with each assay, an estrogen/testosterone dilution series will be added to another set of wells for the determination of a standard dose-response relationship to which the unknown results will be compared. Reporter gene activity in cell extracts will be measured and the EC_{50} (estimated concentration to half-maximal induction) values, calculated from probit analysis. To determine the relative antagonistic potency of a sample extract, cells will be incubated with 5-8 serial dilutions of the sample extract along with a maximal inducing concentration of estrogen or testosterone. Concurrent with each assay, an estrogen/testosterone dilution series with and without a known estrogen/testosterone antagonist will be added to another set of wells for the determination of a standard inhibition curve to which the unknown antagonist results will be compared. Reporter gene activity in cell extracts will be measured and the IC_{50} (estimated concentration to half-maximal inhibition) values, calculated from probit analysis. Comparison of the results to the inhibitory standard curve will allow estimation of the relative estrogen/androgen antagonist activity of the particular test samples.

Following hormonal activity screening, samples with significant activity will be submitted to CAHFS for chemical identification of the active chemicals. It is likely that many water sample extracts will contain too many compounds to enable immediate identification. When this occurs, we will separate the extract into aqueous, semi aqueous, and polar fractions using the methods of Sawyer et al. (2000), re-submit the sample extracts for hormonal activity measurements, and then submit the active fraction(s) to CAHFS for chemical identification. In this manner, the identity of the activating/inhibitory chemical(s) can ultimately be obtained by using the cell bioassays to monitor a classical purification scheme based on differential extraction and column chromatography and instrumental analysis. The relative activity of pure products identified by this approach will also be examined and their contribution to the overall activity of a given sample extract determined.

Task 3: Analytical identification of active compounds.

Identification of the active substances is critical to the success of this project. The California health and food safety laboratory is a State operated diagnostic laboratory that performs chemical identification services for clients throughout California. The toxicology section analyzes a large number of samples on a daily basis (more than 34,000 annually), specializing in pesticides, herbicides, and other toxic substances. The laboratory maintains the Standard Operating Procedure (SOP) for each analytical procedure, and routinely analyzes chemicals according to Good Laboratory Practice (GLP) standards. CAHFS is one of the few laboratories in California capable of screening extracts such as the water samples in this study for the identification of unknown

chemicals. To be able to accommodate the large number of samples anticipated in this study, and to be able to detect steroids, herbicides, and polar pesticides, we are requesting the purchase of a state-of-the-art liquid chromatograph mass spectrometer system to augment the analytical equipment already present in laboratory. The ThermoFinnigan Model TSQ Quantum tandem mass spectrometer and liquid chromatograph provides a new technology, especially important as many new insecticides, herbicides, and fungicides are coming on the market to replace older compounds being phased out by the US EPA.

Water sample extracts will be analyzed by one or several analytical techniques and the chemical(s) present will be identified by mass spectroscopy, and compared to analytical standards. The hormonal activity of the identified chemicals will be confirmed by Denison's laboratory using analytical grade compounds. Because many different chemicals may be responsible for the hormonal activities in different water samples, it is impossible to identify precisely which of the hundreds of methods used by CAHFS will be employed in this project. The methods used will be identified by the SOPs on file in the Laboratory, and new methods developed for this study will be reported in the peer-reviewed literature.

Task 4: Laboratory Screening of Compounds using *Gambusia affinis*.

The Aquatic Toxicology Laboratory at UC Davis maintains facilities for aquaculture of several species of fish. The laboratory routinely conducts toxicology studies with Japanese Medaka (*Oryzias latipes*), and has developed many toxicological and reproductive biomarkers to assess endocrine disruption in this species (Koger et al. 2000, Teh, et al 1998, 2000). Other laboratories in the US and UK have developed similar assessment procedures for trout and other species (Sumpter, et al 1998, Jobling et al 1998, Howell et al 1980, Angus, in press). In this study we propose to use mosquito fish (*Gambusia affinis*) rather than Japanese Medaka, because mosquito fish are widely distributed throughout the Central Valley watershed. *Gambusia* was introduced into California in 1922 (Moyle 1976), and is observed in almost all backwaters and sluggish waters of the Sacramento-San Joaquin River system, including Foothill and mid-elevation reservoirs and ponds. Because mosquito fish are usually considered a beneficial fish, and are used routinely for mosquito abatement, we believe it will be a representative species for the Central Valley watershed, and a suitable species for laboratory evaluation of hormonally active chemicals. Specific biomarkers for *Gambusia* have already been developed by Angus and Howell, although the species is not yet routinely used in our laboratories. This study is designed solely as a laboratory study for evaluation of endocrine disruption in fish, but the biomarkers for *Gambusia* developed in this study will be available for field screening studies in the future, if hormonally active chemicals are identified in the watershed at levels which pose risks.

Preliminary studies on fatty liver disease and reproductive pathology in *Gambusia* have been reported by Teh et al. (2000) identifying the pathological markers which can be used to identify reproductive abnormalities in this species.

Exposure tanks and aquaculture facilities for *Gambusia* will be developed in the first year of this study to be able to replicate the on going studies with Japanese Medaka as described by Koger et al. (2000). Specific modifications must be made in the aquaculture facilities to accommodate this live-bearing species, in order to be able to adequately quantify reproductive impairment and developmental alterations of embryos. Endocrine disruption studies with *Gambusia* are being

conducted with US EPA funding by Angus and Howell at Samford University in Birmingham AL, and these investigators will be consulted on culture techniques, toxicology, and specific biomarkers of reproductive effects.

Gambusia will be exposed to analytical grade compounds using the techniques adapted from the study of Koger, Teh, and Hinton (2000) examining gonadal morphology, fertility, and embryo/larval viability. The numbers of live young, sex ratios of surviving progeny, and histological evaluation of gonads will be used as markers of reproductive function. The specific protein biomarkers vitellogenin and choriogenin have been developed in this laboratory for studies of endocrine disruption in Japanese Medaka, and vitellogenin has been purified and for use as a biomarker in *Gambusia* by Angus et al. During year 1 we will adapt the vitellogenin biomarker techniques for *Gambusia*, and have them ready for full-scale testing during years 2 and 3.

Work in Year 1 will consist of developing aquaculture techniques for *Gambusia*, adapting biomarker and histopathological endpoints, and conducting initial trials with known endocrine disruptors such as the steroid hormone estradiol-17 β . Endocrine disruptors identified in the watershed in year 1 will be tested for activity in *Gambusia* during year 2. Additional compounds that are identified during year 2 will be tested during year 3.

We have developed several sophisticated biomarkers to evaluate reproductive dysfunction and development abnormalities caused by hormonally active chemicals (Koger et al.2000, Werner et al.2000, 2001) and the Aquatic toxicology Lab is in the process of refining real-time polymerase chain reaction (PCR) assays (TaqMan) which provide highly sophisticated evaluations of steroid hormone actions in fish species. (Hall et al. in preparation,). This suite of assays will be perfected by Werner and Hall and will be combined with the pathology assessments by Teh to develop dose-response relationships for adult and juvenile *Gambusia* in laboratory. This laboratory routinely employs the toxicological statistical packages available from the U.S. Fish and Wildlife Service and the US EPA to evaluate the results of laboratory fish exposures.

Task 5: Project Management, Data Evaluation, and Outreach

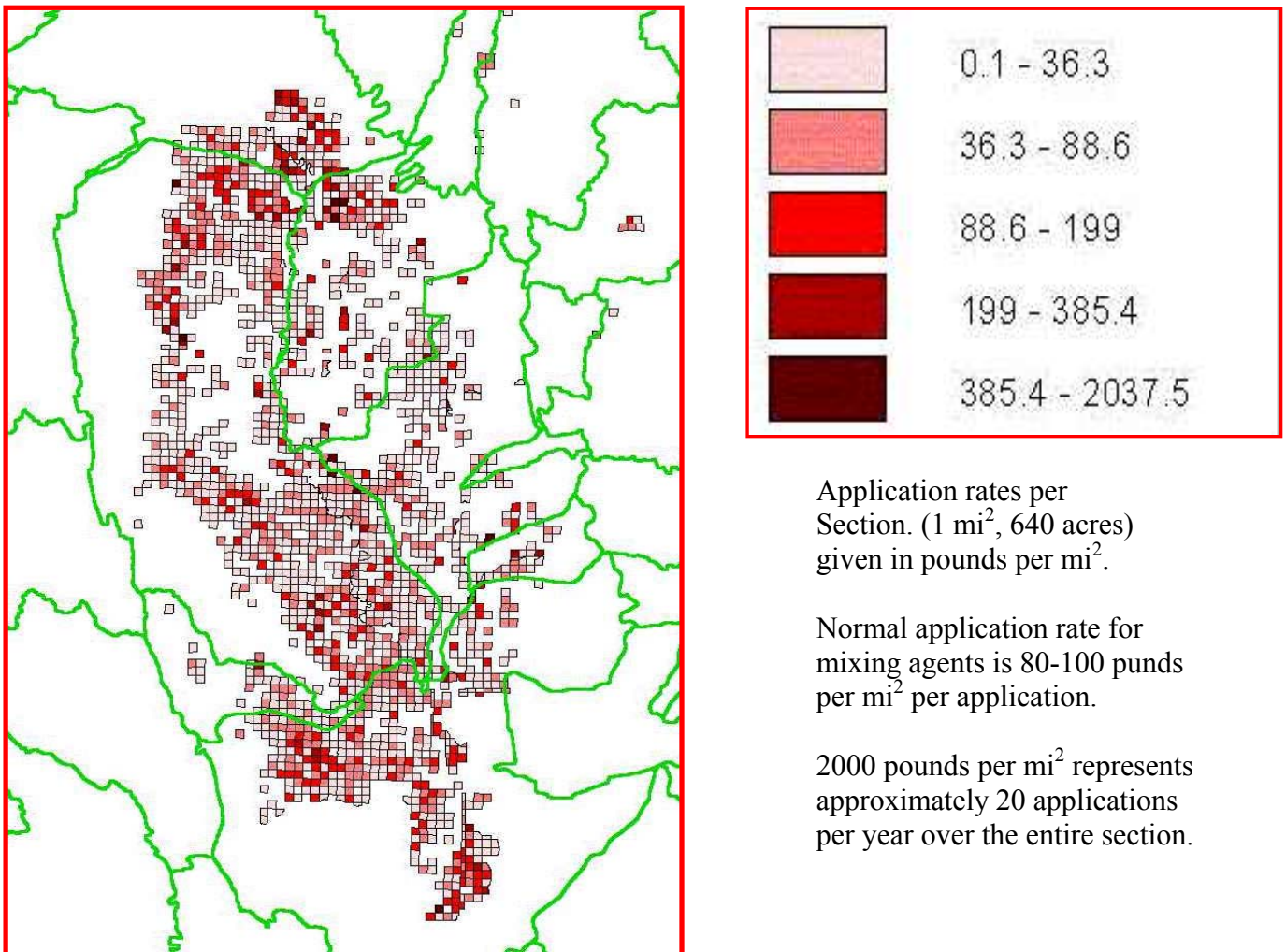
Program management will be divided into five sections for this study.

Section 1). Correlation of data between Tasks: Correlation of water sample concentrations with the quantitative analytical chemistry data from CAHFS will be used to calculate the concentrations of active chemicals present in the water samples collected from throughout the Central Valley. The levels of these chemicals will be compared to the dose-response curves generated by the laboratory exposures of *Gambusia* to determine whether levels of chemicals in the watershed pose any risk to fish. This is a complex relationship, because the levels of chemical required to alter the development of embryos may be different from the levels that cause physiological responses in adult fish. Such factors as the dates of water sampling and the locations will be correlated with known breeding seasons for *Gambusia* and for other species present in the watershed. The uncertainty of chemical effects between species will be evaluated using the techniques developed by the US EPA for evaluating fish exposure to compounds in field studies, as modified by the ECOFRAM committee in 1999 and 2000. (ECOFRAM report, 2000).

Section 2). Adaptive Management of Water Sampling Design:

Correlation of watershed samples with reported pesticide use activity (data from DPR): Those chemicals identified in water samples which are commercially used insecticides, herbicides, or fungicides will be compared with the DPR pesticide use database and will be compared with pesticide use reports provided by applicators to the County agricultural commissioners' office in each county. The geographical extent of upstream chemical use will be evaluated and compared to water samples taken in the initial rounds of water sampling. The most recent annual report of pesticide use from DPR will be used to generate maps of pesticide use, quantified by Township, range, and section. An example map of the usage of alkylphenol poly-ethoxylates is presented as Figure 1. California is the only State that collects data suitable for mapping at this precision. The alkylphenol poly-ethoxylates, and other alkylphenol derivatives, are used as mixing agents in tank formulations of many different pesticides, and are usually considered as inert ingredients. More than 1.75 million pounds of alkylphenols were used in California in 1999 in commercial agriculture (DPR 2000).

Figure 1. Alkylphenol poly-ethoxylate use in the central Sacramento Valley in 1998. Counties of Glenn, Colusa, Yolo, Butte, Sutter, and Yuba are represented. The green boundaries are CA State Water Resources Hydrologic Units.



In some sections, as much as 2000 pounds per square mile were used in 1998. It is unknown what proportion of the residues from these applications runoff into the watershed, and the life span and metabolism of these compounds in the watershed is unknown. The alkylphenols were identified in the UK as one of the primary class of chemicals responsible for fish reproductive impairment (Sumpter et al,1998 Jobling et al.1998), and are thus an important group of compounds to monitor. Maps such as Figure 1 will be prepared for any chemicals identified in Task 3, and the use patterns will be evaluated for planning subsequent water sampling regimens. The DPR database lists more than 800 chemicals, and each can be mapped as in Figure 1, for a single county or for the entire watershed.

The refined water sampling regimen should provide data for the best estimate of the ecological risk posed by chemicals entering the watershed, when that data is compared with the does response results obtained from Task 4.

Section 3). Outreach, Publication, and Information Transfer. Several levels of information transfer will be appropriate in this study. If high concentrations of chemicals are detected in the watershed, they will be reported directly to California DPR, and to the CalFed program to enable the appropriate agencies to monitor or otherwise assess the situation. If any chemicals are detected at levels that cause adverse effects in the *Gambusia* studies, these will be reported in a similar fashion. It is possible that combinations of chemicals may demonstrate significant hormonal activity, without the levels of a single individual chemical rising to a concentration that individually would cause adverse effects. In these circumstances, the sum total of chemicals may pose an environmental risk, and this data will be reported to Agencies for further consultation.

The results of the water sampling studies, the analytical chemistry studies, and the fish screening techniques, will be published in peer review journals, and will be presented at regional and national meetings, such as the annual CalFed symposium, and the Society for Environmental Toxicology and Chemistry.

Section 4). Quality Assurance. Each laboratory will maintain logbooks and records detailing the receipt, storage, and use of each sample. All samples will be identified by printed bar-code labels, affixed to each solid phase extraction cartridge in the field, and logged into the trip log maintained in the GPS software of the laptop computer carried into the field. Bar-code labels will be generated for each sample as it is fractionated and delivered to other laboratories for further analysis. Each laboratory will maintain computer records of its own activities, and will provide computer databases to Fry for central archiving and database management. The Quality Assurance officer within the Department of Animal Science will inspect each laboratory within six months of the initiation of this study, and annually thereafter to certify that all Standard Operating Procedures, safety procedures, and database records are being maintained.

Section 5) Project Management:

The Principal Investigator, Michael Fry, will be responsible for Project Management. Each laboratory identified in Tasks 1-4 will be responsible for maintaining data records, log books, and Quality Assurance documents. Fry will coordinate Task 5, and will be responsible for integration of data and supervision of publications, presentations and information transfer.

The Department of Animal Science will be responsible for maintaining records of the budget, and for inter-Departmental transfers of funds to Departments of Co-PIs. The Co-PI responsible for each Task will be responsible for budget management within the Cost Outline provided in the on-line portion of this proposal. All transactions and budget authority remain with the Regents of the University of California, and all transactions will comply with University regulations. Quarterly Reports and the Final Report will be the responsibility of Fry, with authorships of sections on each Task to be the responsibility of the Co-PIs.

4. Feasibility:

Timeline:

It is anticipated that this project will begin in September 2002. Fry will consult with the Agricultural Commissioners office in each of the 8 Central Valley counties, for recommendations on locations of water sampling to best assess agricultural runoff in each of the counties. The municipal governments of each of the 10 largest metropolitan areas in the Central Valley will be contacted for their cooperation in obtaining water treatment plant outfall samples for measurements. It is anticipated that each of the municipalities will cooperate in this study, although water samples taken from directly downstream of any of the outfalls could be done legally, where the outfalls discharge directly into a river or stream. Sampling sites will generally be located at or adjacent to public highway bridges over agricultural drains or streams throughout the watershed.

We anticipate the beginning water sampling in October 2002. Screening of hormonal activity in Denison's laboratory is anticipated to begin in November 2002 and will continue on a weekly or monthly basis, throughout the study. Turnaround of results from Denison's laboratory can be accomplished within three weeks. The first sample extracts are anticipated to be available for analytical chemistry determinations by CAHFS in January 2003.

CAHSF has an ongoing diagnostic toxicology program with a throughput of more than 2500 samples per month. Samples for this study will be assigned to a halftime postgraduate researcher dedicated to this project for sample preparation and analysis. The Co-Principal Investigator Puschner, and the laboratory analyst Elizabeth Tor will supervise analyses. Many of the sample extracts should be routine for chemical analysis using existing standard operating procedures in the laboratory. Some samples may require method development, and the more polar compounds, including herbicides and fungicides, as well as steroid metabolites from municipal wastewater outfalls, will be kept in storage until the new Finnegan liquid chromatograph/mass spectrometer is purchased and installed early in 2003.

Is anticipated that chemicals will be identified from water samples and available for re-testing in Denison's hormone activity assays during the spring of 2003. Chemicals with confirmed hormonal activity will be available to the Aquatic Toxicology Laboratory for screening with *Gambusia* by September 2003, the beginning of Year 2 of this project.

During Year 1, the Aquatic Toxicology Lab, including Drs. Teh, Werner, and Hall, will develop the aquaculture methods for *Gambusia*, and adapt current biomarker techniques to *Gambusia*. Drs. Angus and Howell at Samford University will be contacted directly, or through U.S. EPA, to obtain the vitellogenin immuno-reagents specific for *Gambusia*. The biomarker techniques already perfected in the Aquatic Toxicology Laboratory should be successfully adapted for use with *Gambusia* during Year 1, so that the laboratory will be prepared to begin the screening of pure chemicals at the beginning of Year 2.

The data generated by hormone activity measurements and analytical chemistry identification of specific chemicals will be compared with DPR pesticide use lists beginning in the second half of year 1, and will be used to adapt the sampling regimen for water samples in years 2 and 3. This process of adaptive management for field sampling and selection of chemicals for laboratory fish screening will continue during years 2 and 3. The selection of the active chemicals for screening in *Gambusia* studies will be important decisions, made in consultation with California DPR, the CalFed program, the U.S. EPA endocrine disruptor program (Robert Kavlock), and other scientific colleagues.

We anticipate that a larger number of hormone active chemicals will be identified in the watershed than will be possible to be tested by the Aquatic Toxicology Laboratory, because of the time labor-intensive nature of the fish screening and biomarker assays. We will select the most significant compounds for fish analysis. We plan to conduct toxicological assessments with only 12-15 compounds during the course of this study. However, generating geographical information, hormone assessments, chemical analysis, and fish dose-response toxicology information on a dozen chemicals will be of enormous benefit to the state of California, and to the scientific community at large.

5. Performance measures:

Project performance evaluation and milestones have been presented throughout the descriptions and of the individual tasks. Each water sample will be handled individually, and results from each task will be used in the determination of subsequent analyses. The results obtained in tasks 2,3, and 4, will be used to make decisions as to where subsequent sampling will be conducted in Task 1, illustrating that the entire project has internal performance measures and milestones to keep the project on track, and clearly focused.

Quarterly reporting: Program and fiscal records will be submitted to CalFed in January, April, July, and October of each of the three years of project duration.

6. Data Handling and Storage:

Each laboratory will maintain logbooks and records detailing the receipt, storage, and use of each sample. All samples will be identified by printed bar-code labels, affixed to each solid phase extraction cartridge in the field, and logged into the trip log maintained in the GPS software of the laptop computer carried into the field. The trip-log and data entries will be archived for each sample collection trip to positively identify the time and location of each sample collection. Bar-code labels will be generated for each sample as it is fractionated and delivered to other laboratories

for further analysis. Each laboratory will maintain computer records of its own activities, and will provide computer databases to Fry for central archiving and database management.

7. Expected Products/Outcomes

The results of the water sampling studies, the analytical chemistry studies, and the fish screening techniques will be published in peer review journals, and will be presented at regional and national meetings, such as the annual CalFed symposium, and the Society for Environmental Toxicology and Chemistry. Several other levels of information transfer will be appropriate in this study. If high concentrations of chemicals are detected in the watershed, they will be reported directly to California DPR, and to the CalFed program to enable the appropriate agencies to monitor or otherwise assess the situation. If any chemicals are detected at levels that cause adverse effects in the *Gambusia* studies, these will be reported in a similar fashion. It is possible that combinations of chemicals may demonstrate significant hormonal activity, without the levels of a single individual chemical rising to a concentration that individually would cause adverse effects. In these circumstances, the sum total of chemicals may pose an environmental risk, and this data will be reported to Agencies for further consultation.

8. Work Schedule

It is anticipated that this project will begin in September 2002.

We anticipate the beginning water sampling in October 2002. S

Screening of hormonal activity in Denison's laboratory is anticipated to begin in November 2002 and will continue on a weekly or monthly basis, throughout the study.

The first sample extracts are anticipated to be available for analytical chemistry determinations by CAHFS in January 2003.

The Finnegan liquid chromatograph/mass spectrometer is purchased and installed early in 2003.

Chemicals with confirmed hormonal activity will be available to the Aquatic Toxicology Laboratory for screening with *Gambusia* by September 2003, the beginning of Year 2 of this project.

During Year 1, the Aquatic Toxicology Lab, including Drs. Teh, Werner, and Hall, will develop the aquaculture methods for *Gambusia*, and adapt current biomarker techniques to *Gambusia*.

The laboratory will begin the screening of pure chemicals at the beginning of Year 2.

The data generated by hormone activity measurements and analytical chemistry identification of specific chemicals will be compared with DPR pesticide use lists beginning in the second half of year 1, and will be used to adapt the sampling regimen for water samples in years 2 and 3.

B. The Applicability to CalFed ERP and Science Program gGoals

1. ERP, Science Program, and CVPIA Priorities:

The need for a study assessing hormonally active chemicals in the watershed is specifically described in the program goals of the Ecosystem Restoration Program under Strategic Goal 6: Sediment and Water Quality. The strategic goal states "**synthetic compounds used in medicines,**

cosmetics, and as biocides are widespread in many aquatic environments and have been linked with effects on reproduction (endocrine disruption) elsewhere. Yet these substances have never been studied in the Bay-Delta...Moreover, there is no comprehensive understanding of the risk that contaminants might pose to the health of individuals and populations in the estuary or upstream of the tidal portion of the ecosystem”.

The ERP Proposal Solicitation Package specifically lists endocrine disruptors as one of the priorities under MR-5, the Multi-Regional priority for Water Quality, Subsection: Other Pollutants.

In addition to Multi-regional priorities, the priorities for the Sacramento, San Joaquin, and Bay Regions all list endocrine disruptors as a priority for study. The Bay-Region priority BR-6) Restoration of shallow water, local stream, and riparian habitat... states **“Begin studies of “emerging” chemicals, including pharmaceuticals, cosmetic products and estrogens that have apparent impacts on animal reproduction elsewhere. Clarification of distributions (time and space) and potential effects on local fauna/flora are needed (Strategic Goal 6, other pollutants)”.**

This study is specifically designed to address those contaminants and uncertainties, and to provide a geographic distribution and risk assessment of endocrine disruptors throughout the freshwater portion of the Central Valley watershed.

2. Relationship to other ecosystem restoration projects

No specific study is being conducted on endocrine disruptors in the central Valley Watershed, or in other parts of California at this time. A pilot study of fish was initiated by the USGS, NAQWA program, and that study discovered significant reproductive differences between populations of fish in the San Joaquin watershed, but did not correlate the differences to specific chemicals. An on-going study in at Lake Mead, NV, has demonstrated highly significant reproductive dysfunction in fish associated with endocrine disruptors in outfalls and lake water (Snyder et al 2001a,b).

This project will work closely with the USGS proposed CALFED project titled “Pyrethroid Insecticides: Analysis, Occurrence, and Fate in the Sacramento and San Joaquin Rivers and Delta”, if that project is funded. New pyrethroid insecticides pose enormous difficulties in detection, quantification, and toxicity estimates, and that study is specifically designed to address the pyrethroid problem. We have conferred with Kathy Kuivila, a participant in that proposal, and have offered to test samples of chemicals detected by that study for hormone activity, and to work with their program in coordination of sample collection and analysis.

3. Requests for Next-Phase Funding: Not applicable to this proposal.

4. Previous Recipients of CALFED Program Funding:

CALFED 99-N07 (Chronic Toxicity of Environmental Contaminants in Sacramento Splittail (*Pogonichthys macrolepidotus*): A Biomarker Approach: Hung, Teh, and Davis. 65% complete. We have completed two seasons of field sampling and three laboratory studies. Two papers have been submitted for publication. In the final year of this project, we will focus on analyzing field samples for organochlorines and heavy metals and compare the chemical data to the biochemical and histopathological indicators. Currently, we are working on the dietary exposure of juvenile

splittail to various concentrations of Selenium.

CAIFED-B81650. (Role of contaminants in the decline of Delta Smelt in the Sacramento-San Joaquin Estuary). Bennett, Teh, and Anderson. 90% complete. This project has developed tools for quantifying the potential effects of poor food supply and contaminant exposure on the growth and survival of individual delta smelt collected in the IEP abundance surveys. Final report to be submitted to CALFED in October 2001.

5. System-wide ecosystem benefits

This project is designed to be responsive in sampling strategy, so that if chemical activity is discovered in one area of the watershed, sampling will be initiated in other parts of the watershed having the same chemical use patterns. Identification of specific chemicals will enable regulators to control application and runoff in other, unstudied, areas where similar problems might be expected. This will not only be applicable to watersheds Statewide, but throughout the nation.

6. Additional Information for Proposals Containing Land Acquisition: Not applicable to this proposal.

C. Qualifications

The individual researchers in this team have national and international reputations for work on endocrine disruptors and environmental toxicology, and analytical chemistry.

Dr. Michael Fry, Ph.D. is wildlife toxicologist in the Department of Animal Science, UC Davis, with research interests in endocrine disruptor effects on fish and wildlife. He has been a National Academy of Science Panel member and co-author of the National Academy book "Hormonally Active Agents in the Environment". He has served on US EPA committees for development of screening methods for endocrine disruptors, and has been a member of the OECD panel on revising avian toxicology testing guidelines. He has served on the US EPA Ecological Committee for FIFRA Risk Assessment Methods (ECOFRAM), with emphasis on incorporating endocrine disruptor testing methods into pesticide regulations. Fry has more than 60 peer-reviewed publications and dozens of national presentations on wildlife toxicology, emphasizing field studies of pollutants and laboratory confirmation of physiological effects of a diverse range of chemicals with endocrine disruptive effects.

Dr. Michael Denison, Ph.D. is a Professor in the Department of Environmental Toxicology at UC Davis, and is an internationally respected scientist specializing in development of assay techniques for endocrine disruptors and organohalogen compounds. Denison has 78 peer-reviewed publications in environmental toxicology and is an Editor for Chemosphere: POPs and Dioxin section, an Editorial Board Member of: Chemical Research In Toxicology, the Journal of Biochemical and Molecular Toxicology, and Environmental Toxicology and Pharmacology.

Dr. Birgit Puschner, DVM, Ph.D. is Clinical Toxicologist and Director of the Toxicology Section of the California Animal Health and Food Safety Laboratory, at UC Davis, and an Assistant

Professor in the Department of Clinical Veterinary Toxicology at the UC Davis School of Veterinary Medicine. Dr. Puschner supervises the analytical chemistry laboratory and ion chromatograph that will be used in this study. She is a Certified Veterinary Toxicologist, American Board of Veterinary Toxicology, and a member of the American Association of Veterinary Laboratory Diagnosticians, California Veterinary Medical Association, Sacramento, the Society of Toxicology, and the Deutsche Veterinärmedizinische Gesellschaft. She has 13 peer-reviewed publications, several chapters of books, and numerous presentations.

Michael L. Johnson, Ph.D. is the Director, Lead Campus Program in Ecotoxicology, UC Toxic Substances Research & Teaching Program, and an Associate Researcher, John Muir Institute of the Environment, UC Davis. He is Interim Director of the Center for Aquatic Biology and Aquaculture (CABA) at UC Davis. The CABA maintains facilities for the culture and study of both laboratory and wild fishes. The facilities will be used initially for the culture of *Gambusia*, and could be used for studies of native fish in subsequent years. Dr Johnson has been the Principal Investigator in several major grants assessing watersheds and toxic substances in California. He is a Member, American Society of Mammalogists; Ecological Society of America; American Society of Naturalists; Society of Environmental Toxicology and Chemistry; Society for Human and Ecological Risk Assessment; Society for Risk Analysis, Society for Conservation Biology, American Association for the Advancement of Science.

Dr. Swee J. Teh, is a member of the Research Toxicology and Pathology faculty at UC Davis, Dept. of Anatomy, Physiology and Cell Biology and has over 14 years of extensive field and laboratory research experience in ecotoxicology and biomarker studies. His research interests are in the fields of developmental biology, nutrition, toxicology and pathology with special emphasis on adverse health, reproductive, and embryonic developmental effects of environmental endocrine disruptors and contaminants in invertebrate, fish and shellfish populations. He has publications, and travels nationally and internationally presenting talks and workshops in this area.

Dr. Ingeborg Werner, Ph.D. UC Davis, Dept. of Anatomy, Physiology and Cell Biology, School of Veterinary Medicine. Dr. Werner holds a master's degree in limnology from University of Freiburg, Germany, and a doctoral degree in ecotoxicology ('magna cum laude') from University of Mainz, Germany. She has 10 years of experience in biomarker research and aquatic toxicity testing, and is part of the research faculty at UC Davis. Her research interests focus on sublethal effects of pollutants in aquatic invertebrates and fish, and the development and application of toxicity tests using chronic endpoints and cellular and biochemical biomarkers at various levels of organisation. Other components of her work include aquatic monitoring studies to assess pesticide toxicity in the Delta, impact and efficacy of alternative pest control methods in the Sacramento/San Joaquin watershed, toxicity of MTBE to freshwater organisms, and toxicity of stormwater runoff in California urban areas.

Linda Hall holds a Master's degree in Toxicology from San Jose State University, and will receive her Ph.D from UC Davis in Ecological Toxicology in June (2002). Since 1988 she has been employed as an Environmental Scientist at Lawrence Livermore National Laboratory, Health and Ecological Assessment Division, where she conducted research in human health risk assessment. Her doctoral research at UC Davis has focused on techniques to identify and evaluate novel endocrine disrupting substances using the fish medaka (*Oryzias latipes*) as a model organism. Specifically, her research interests have incorporated techniques such as

ligand binding assays to identify compounds that competitively displace estradiol from its receptor; the design and conduct of experiments with *O. latipes* to identify novel reproductive toxins that possess estrogenic action; application of electrophoresis and Western blotting techniques to assess the ability of environmental contaminants to induce expression of estrogen-regulated proteins; and application of real time polymerase chain reaction (PCR) to quantify contaminant-induced changes in expression of the estrogen receptor and cytochrome P450 aromatase in brain and reproductive tissues of medaka.

Elizabeth Tor, M.S. Agricultural and Environmental Chemistry, University of California, Davis, is a Senior Analytical Chemist, Staff Research Associate IV, Organic Residue Group Leader, at the California Animal Health and Food Safety Laboratory System (CAHFS), Toxicology Laboratory, UC Davis, Davis, CA. She is Responsible for daily operation of the Organic Residue Group. Supervise the work of two chemists (Staff Research Associates II) and other lab personnel analyzing organic contaminants in a great variety of samples of animal and plant origin. She has 11 peer-reviewed publications.

D. Cost:

1. Budget: budget is included in the Web forms.
2. Cost Sharing: No cost sharing is proposed.

E. Local Involvement:

The sampling plan for this project begins with consultation with the Agricultural Commissioners' office in each of the eight Central Valley Counties, for recommendations on locations of water sampling to best assess agricultural runoff in each of the counties. The municipal governments of each of the 10 largest metropolitan areas in the Central Valley will be contacted for their cooperation in obtaining water treatment plant outfall samples for measurements.

We plan to sample major agricultural drains and their tributaries, convergence points of agricultural drains and creeks or rivers, and municipal water treatment plant outfalls. Prior to sampling we will confer with municipal water treatment facilities, and with the agricultural commissioners' office in Sutter, Colusa, Yolo, Sacramento, Solano, San Joaquin, Stanislaus, and Merced Counties. In Year 2, we plan to increase the geographical scope of water sampling, to include parts of Tehama, Glenn, Butte, Yuba, Madera, Contra Costa and Fresno Counties, and possible inclusion of parts of Placer, El Dorado, and Amador Counties.

F. Compliance with Standard Terms and Conditions:

The University of California Office of the Vice Chancellor for Research has reviewed this proposal and the CALFED terms and conditions. A signed letter of disclaimer stating the position of the University has been FAXed with the Signature page stating the University's position on compliance.

G. Literature Cited:

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