Water quality effects of pesticides used in orchard agriculture - Part 2: Aquatic fate and effects of particle-sorbed pyrethroids

Project Information

1. **Proposal Title:**

   Water quality effects of pesticides used in orchard agriculture - Part 2: Aquatic fate and effects of particle-sorbed pyrethroids

2. **Proposal applicants:**

   Donald Weston, University of California, Berkeley

3. **Corresponding Contact Person:**

   Donald Weston
   University of California, Berkeley
   1301 S. 46th St., Bldg. 112 Richmond, CA 94804
   510 231-5626
   dweston@uclink4.berkeley.edu

4. **Project Keywords:**

   Ag/Urban Runoff
   Pesticides
   Sediment quality

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:	38.100
Longitude:	121.600
Datum:	

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

Project includes both laboratory and field work. Field components will be done at sites in both Sacramento and San Joaquin watersheds that will be identified after initiation of project.

10. **Location - Ecozone:**

    3.3 Chico Landing to Colusa, 3.4 Colusa to Verona, 8.1 Feather River, 12.1 Vernalis to Merced River, 13.1 Stanislaus River, 13.2 Tuolumne River, 13.3 Merced River, Code 15: Landscape

11. **Location - County:**

    Butte, Glenn, Merced, Stanislaus, Sutter, Yolo

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:**

    9

15. **Location:**

    California State Senate District Number: 9

    California Assembly District Number: 14

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:	10.0
Total State Funds:	1,925,430
Federal Overhead Rate:	50.4
Total Federal Funds:	2,452,462

b) Do you have cost share partners already identified?

Yes

If yes, list partners and amount contributed by each:

U.S. Army Corps of Engineers 333,368

Southern Illinois University 40,000

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.

99-N08	**Assessment of pesticide effects on fish and their food resources in the Sacramento-San Joaquin Delta**	**ERP**
97-C12	**Alternative practices for reducing pesticide impacts on water quality**	**ERP**
99-N07	**Chronic toxicity of environmental contaminants in Sacramento splittail: a biomarker approach**	**ERP**

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)

Blair Siegfried	**Univ. Nebraska, Dept. Entomology**	**402-472-8714**	**bsiegfried1@unl.edu**
Joel Coats	**Iowa State Univ., Dept. Entomology**	**515-294-4776**	**jcoats@iastate.edu**
Debra Denton	**US EPA, Region 9**	**415-744-1919**	**denton.debra@epamail.epa.gov**

21. **Comments:**

Environmental Compliance Checklist

Water quality effects of pesticides used in orchard agriculture - Part 2: Aquatic fate and effects of particle-sorbed pyrethroids

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 research project with no action in the field other than sample collection.

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
   Xnone

   NEPA
   -Categorical Exclusion
   -Environmental Assessment/FONSI
   -EIS
   Xnone

   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.

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: Farms yet to be identified — Required

6. **Comments.**

Land Use Checklist

Water quality effects of pesticides used in orchard agriculture - Part 2: Aquatic fate and effects of particle-sorbed pyrethroids

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?**

   Yes

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).

   Research only

4. **Comments.**

   Field work will involve collection of samples in public waters and on private land at at least 2 orchards. The specific farms will be identified shortly after project initiation. We have a long history of similar research at many farms throughout the region, and anticipate no difficulty gaining access.

Conflict of Interest Checklist

Water quality effects of pesticides used in orchard agriculture - Part 2: Aquatic fate and effects of particle-sorbed pyrethroids

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 Weston, University of California, Berkeley

Subcontractor(s):

Are specific subcontractors identified in this proposal? Yes

If yes, please list the name(s) and organization(s):

Michael Lydy	Southern Illinois University
Inge Werner	Univ. California, Davis
Swee Teh	Univ. California, Davis
Shirley Gee	Univ. California, Davis
Frank Zalom	Univ. California, Davis
None	None
None	None
None	None
None	None

Helped with proposal development:

Are there persons who helped with proposal development?

No

Comments:

None

Budget Summary

Water quality effects of pesticides used in orchard agriculture - Part 2: Aquatic fate and effects of particle-sorbed pyrethroids

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.

State 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	Field studies	1925	46679	9578	1500	9300	123227	3500	0	193784.0	8984	202768.00
2	Invert. toxicity/bioaccumulation	2200	51012	9708	2500	21000	0	27000	0	111220.0	8200	119420.00
3	Toxicokinetics	0	0	0	0	0	66994	0	0	66994.0	0	66994.00
4	Fish toxicity	0	0	0	0	0	223856	0	0	223856.0	0	223856.00
5	Pesticide mixtures	0	0	0	0	0	0	0	0	0.0	0	0.00
6	Project management	440	13657	1933	700	1500	22285	0	0	40075.0	1779	41854.00
		4565	111348.00	21219.00	4700.00	31800.00	436362.00	30500.00	0.00	635929.00	18963.00	654892.00

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	Field studies	1910	48113	9626	3000	8700	113567	0	0	183006.0	6713	189719.00
2	Invert. toxicity/bioaccumulation	2220	53565	10172	2500	21000	0	0	0	87237.0	8493	95730.00
3	Toxicokinetics	0	0	0	0	0	53711	0	0	53711.0	0	53711.00
4	Fish toxicity	0	0	0	0	0	218199	0	0	218199.0	0	218199.00
5	Pesticide mixtures	0	0	0		0	72987	0	0	72987.0	0	72987.00
6	Project management	440	14341	2030	700	1500	22970	0	0	41541.0	1848	43389.00
		4570	116019.00	21828.00	6200.00	31200.00	481434.00	0.00	0.00	656681.00	17054.00	673735.00

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	Field studies	850	22018	4187	2500	4500	41918	0	0	75123.0	3321	78444.00
2	Invert. toxicity/bioaccumulation	2140	52231	9554	2500	17000	0	0	0	81285.0	7906	89191.00
3	Toxicokinetics	0	0	0	0	0	55891	0	0	55891.0	0	55891.00
4	Fish toxicity	0	0	0	0	0	230954	0	0	230954.0	0	230954.00
5	Pesticide mixtures	0	0	0	0	0	76083	0	0	76083.0	0	76083.00
6	Project management	960	26700	5261	700	1500	28864	0	0	63025.0	3194	66219.00
		3950	100949.00	19002.00	5700.00	23000.00	433710.00	0.00	0.00	582361.00	14421.00	596782.00

Grand Total=1925409.00

Comments.

Budget Justification

Water quality effects of pesticides used in orchard agriculture - Part 2: Aquatic fate and effects of particle-sorbed pyrethroids

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

UCB - Weston: 3675 hr over 3 yr UCB - Grad. student: 4200 hr over 3 yr UCB - Staff Res. Assoc.: 3760 hr over 3 yr UCB - Undergrad. Lab. Asst.: 1470 hr over 3 yr SIU - Post-doctoral Researcher; 5940 hr over 3 yr SIU - Graduate students (2); 3168 hr each over 3 yr UCD - Werner; 2592 hr over 3 yr UCD - Teh; 2784 hr over 3 yr UCD - Postgraduate Researcher VI; 4800 hr over 3 yr UCD - Postgraduate Researcher III; 8640 hr over 3 yr UCD - Student Postgrad. Res. I; 5760 hr over 3 yr UCD - Staff Research Assoc. I; 1280 hr over 3 yr UCD - Lab. Asst. III; 2880 hr over 3 yr UCD - Lab. Asst. II; 2112 hr over 3 yr UCD - Undergrad.; 6720 hr over 3 yr

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

UCB - Weston: $7256/mo + 5%/yr UCB - Grad. student: $2773/mo + 5%/yr UCB - Staff Research Assoc.: $3638/mo + 5%/yr UCB - Undergrad. Lab. Asst.: $2100/mo + 5%/yr SIU - Post-doctoral Researcher: $2700/mo + 5%/yr SIU - Graduate students: $1,158/mo + 5%/yr UCD - Werner: $5503/mo + 5%/yr UCD - Teh: $5504/mo + 5%/yr UCD - Post-grad. Researcher VI: $3365/mo + 5%/yr UCD - Post-grad. Researcher III: $3075/mo + 5%/yr UCD - Student Post-grad. Res. I: $2574/mo + 5%/yr UCD - Staff Res. Assoc. I: $2653/mo + 5%/yr UCD - Lab. Asst. III: $2914/mo + 5%/yr UCD - Lab. Asst. II: $2181/mo + 5%/yr UCD - Undergrad.: $1400/mo + 5%/yr

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

UCB - Faculty: 17% UCB - Grad. student: 3% + $4440 tuition & health/yr UCB - Staff Res. Assoc.: 23% UCB - Undergrad. Lab. Asst.: 3% SIU - Post-doctoral Res.: 12.08% + $912/mo health SIU - Grad. students: No charge, SIU covers UCD - Faculty: 17% UCD - Post-grad. Res.: 23% UCD - Student Post-grad. Res.: 3% UCD - Staff Res. Assoc.: 25% UCD - Lab. Asst.: 23% UCD - Undergrad.: 3%

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

UCB - National scientific meeting; 2/yr; $1500/trip; present project results. SIU - Round-trip Carbondale IL to Berkeley CA; 2/yr; $750/trip: coordination with project personnel SIU - National scientific meeting; 1/yr; $1500/trip; present project results. UCD - National scientific meeting; 3/yr (1 per P.I.); $1200/trip; present project results

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

UCB - Office: $2000-3000/yr UCB - Lab.: $20,000/yr (radiochemicals = half of total) UCB - Computing: $1500-3000/yr UCB - Field: $3,000-9,000/yr SIU - Office: $2500-$3500/yr SIU - Lab.: $11,000-$14000/yr SIU - Computing and field: 0 UCD - Office: $3000/yr UCD - Lab.: $18000/yr UCD - Computing: $1800/yr UCD - Field: $2000/yr in yr 1,2 only

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.

UC Davis subcontract = $943,514 if state funds; $1,265,484 if fed. funds. Southern Illinois University subcontract = $407,991

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.

UCB - Hydrolab: $3500, Scintillation counter: $27,000 SIU - None UCD - Ultracold freezer: $8900

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 presentatons, reponse to project specific questions and necessary costs directly associated with specific project oversight.

For preparation of quarterly/annual/final report, QA/QC plan, presentations. Costs shown are direct cost only. UCB - $52,000 SIU - $14,500 UCD - $48,000

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.

UCB - If funds are provided by a California state agency the indirect cost rate at UC Berkeley is 10% of total direct costs excluding equipment and subcontracts. Subcontracts are assessed an indirect cost of 10% on the first $25,000, and nothing thereafter. These subcontract-associated indirect costs have arbitrarily been placed in Task 1, Year 1. No UCB indirect charge on funds passed on to UC Davis. If the source of funds is a federal agency, the applicable indirect cost rate is 50.4% on the same modified total direct costs and applied in the same way to subcontracts. CALFED's choice of fund source will impact both the indirect costs on the project as a whole, as well as the Services and Consultants line item, since a subcontract is being issued to UC Davis, and they too have different rates for state and federal sources. The budget form has been filled out assuming state funds. If funded from federal sources, the UC Davis subcontract value will increase from $943,514 to $1,265,484, and the overall project cost will increase from $1,925,409 to $2,451,147. SIU - SIU's federally-negotiated indirect cost rate is 41% applied to total direct costs minus equipment, and is independent of source of funds. UCD - If the source of funds is a California state agency, the indirect cost rate is 10% applied to total direct costs minus equipment and student fees; If the funds are of federal origin, the rate is 48.5% applied to same basis.

Executive Summary

Water quality effects of pesticides used in orchard agriculture - Part 2: Aquatic fate and effects of particle-sorbed pyrethroids

Throughout the 1990s there has been a gradual shift away from organophosphate pesticides and toward pyrethroids, and this trend is anticipated to accelerate. Because of their hydrophobicity of pyrethroids, terrestial soils and aquatic sediments are likely to be a long-term reservoir for residues, and the primary vector for long-range transport from urban or agricultural points of use. No information exists on the levels of pyrethroids in the sediments of the Sacramento-San Joaquin watershed, and even if it did, the toxicological data needed to assess risk of particle-sorbed residues is lacking. We propose a 3 yr field and laboratory effort to determine the presence and persistence of pyrethroid residues in sediments, assess risk to invertebrates of trophic importance to at-risk fish species, and examine the potential for adverse effects in splittail through chronic dietary exposure. At at least two farms we will study the persistence of pyrethroids in soils, and thus the potential for multiple toxicity pulses with later rain events. We will quantify the toxicity of runoff by in situ toxicity tests, an approach that has been underutilized in the Central Valley but well-suited to pyrethroids. We will also do broad surveys to quantify pyrethroid levels in sediments in highly agriculture- and urban-affected watercourses as well as larger sloughs and mainstem rivers. In order to evaluate the ecological relevance of these data, laboratory studies will evaluate the bioavailability of sediment-bound pyrethroids by both standard bioaccumulation testing and a novel in vitro digestive fluid extraction approach. We will assess the toxicity of sediment-bound residues to 5 benthic invertebrate species, most of which are important prey for salmon, sturgeon or splittail. We will assess the ability of these invertebrate taxa to metabolize pyrethroids, or potentially retain the toxicologically active compound for trophic transfer to fish predators. Finally we will examine the potential for dietary uptake of pyrethroids to elicit histopathological disorders and biochemical indications of stress in the Sacramento splittail, and use these biochemical indicators for field assessment of pesticide exposure. Our study will provide the basis for resource management agencies to assess the risks of this emerging pesticide class on ecosystems within the Sacramento and San Joaquin watersheds, and mitigate potential impacts on on-going restoration efforts.

Proposal

University of California, Berkeley

Water quality effects of pesticides used in orchard agriculture - Part 2: Aquatic fate and effects of particle-sorbed pyrethroids

Donald Weston, University of California, Berkeley

Water Quality Effects of Pesticides used in Orchard Agriculture – Part 2: Aquatic Fate and Effects of Particle-sorbed Pyrethroids

A. PROJECT DESCRIPTION

1. Problem

The agriculture industry and urban population have relied heavily on organophosphate (OP) pesticides for three decades. Numerous studies (Kuivala & Foe 1995, many others) have demonstrated that the timing and mode of application and the relative water solubility of OPs have led to runoff of residues from the application site with pulses of toxicity moving through the Sacramento and San Joaquin Rivers. In recent years, the incidence of toxicity to water column organisms appears to be declining. Our own studies showed no mortality of *Ceriodaphnia dubia* in the Sacramento and San Joaquin Rivers last winter despite frequent sampling. These observations may be related to a shift in pesticide use patterns that, until recently, has gone largely unnoticed by water resource managers. Pyrethroid pesticides have been used with increasing frequency as OP use has declined. In 1992, approximately 47% of the Central Valley's almond acreage was treated with OPs, and only about 3% with pyrethroids. By 1998, the acreage treated with OPs had decreased to 18%, and the proportion treated with pyrethroid had increased to 20% (Epstein et al. 2000). With EPA's recent decisions to ban residential use of the most popular OPs, diazinon and chlorpyrifos, and to further restrict agricultural use of chlorpyrifos, pyrethroids use is likely to increase further.

The two most heavily used agricultural pyrethroids in California are permethrin and esfenvalerate with smaller amounts of cypermethrin, bifenthrin, cyfluthrin, and *lambda*-cyhalothrin. These pesticides present several difficulties for those involved in protecting water quality. First, they are extremely toxic to some aquatic life. Fish, in particular, are far more sensitive to pyrethroids than to OPs. Secondly, the information available on off-site movement, fate and biological effects of pyrethroids is more limited than for the OPs. Thirdly, they present substantial analytical problems. No commercial contract laboratories in the region analyze for pyrethroids, and while research labs are able to quantify pyrethroid concentrations down to a 5-50 ppt range, they are toxic to the most sensitive species at even lower concentrations. Finally, pyrethroids are less soluble than OPs and are therefore more likely to be found on suspended and bedded sediments than in the dissolved phase. Resource management agencies in the Central Valley have traditionally focused on dissolved pesticides. Particulate matter has routinely been filtered out and discarded prior to analysis of water samples. No studies have examined sediments for pyrethroid residues.

2. Justification

We believe that proper management of the use of pyrethroids or any other pesticide requires an approach that includes optimizing application on the farm, minimizing off-site movement from agricultural lands, and understanding the fate and effects of the pesticide in surface waters, i.e., "from tree to sea". Therefore, we are coordinating our efforts with those of a companion proposal from Zalom et al. of UC Davis (Water Quality Effects of Pesticides used in Orchard Agriculture - Part 1: Evaluating Management Alternatives and Off-site Movement). Part 1 will examine alternative management practices, improved application technology, and off-site movement of residues, while our proposed work (Part 2) will help to understand the fate of those residues and effects on aquatic biota once they leave agricultural lands (Figure 1).

Pyrethroids that reach aquatic systems due to spray drift following agricultural application are initially in the dissolved phase and can be acutely toxic to water column organisms (Samsoe-Petersen et al. 2001). However, this dissolved phase pulse is likely to be short-lived and localized. For example, esfenvalerate has been shown to have a half-life in the water column of only 10 hr (Fairchild et al. 1992). Particle adsorption results in a rapid reduction in toxicity for water column

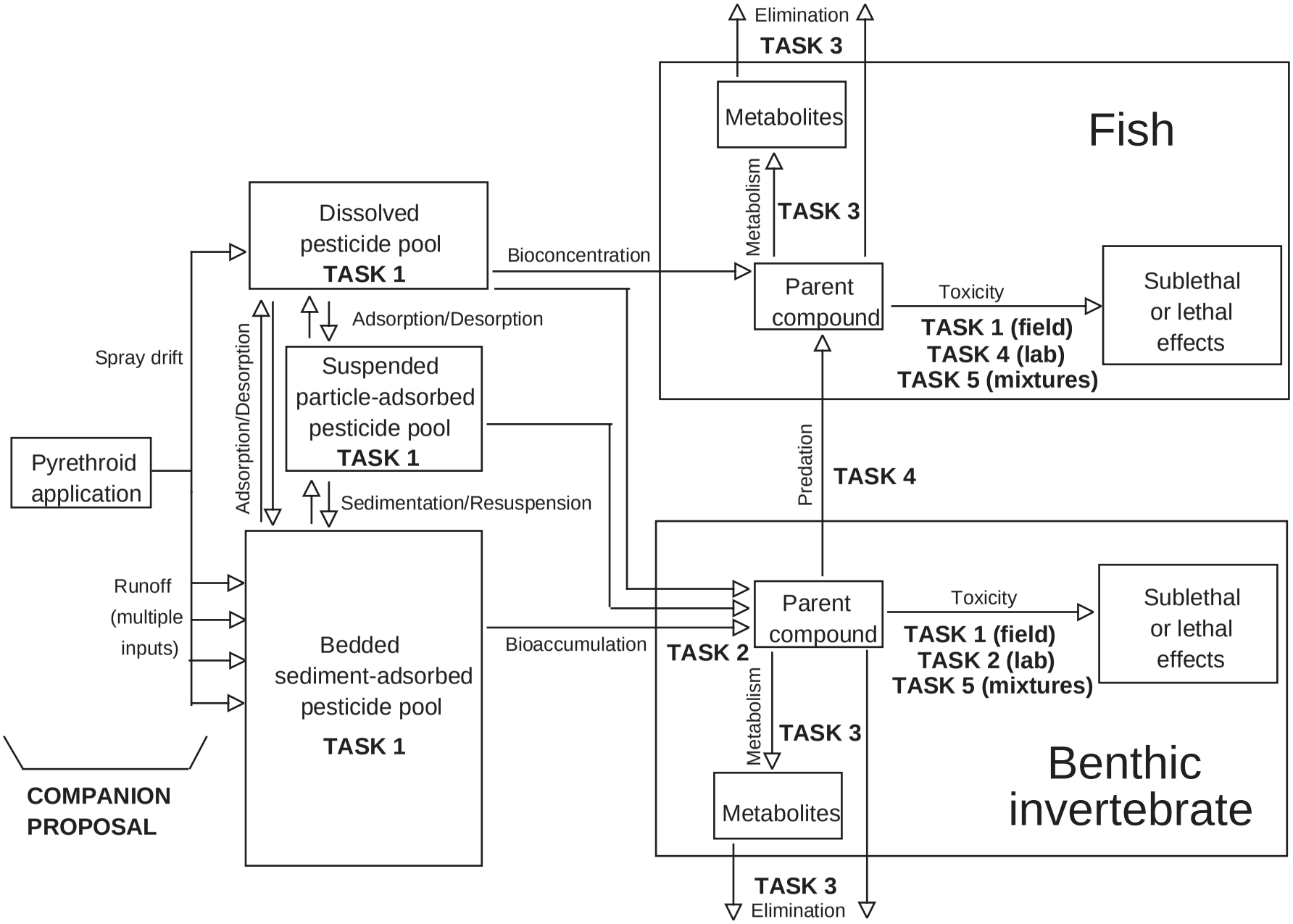

Figure 1. Conceptual model linking elements of pyrethroid environmental fate and biological effects relevant to proposal.

organisms (Crossland 1982), but it increases the risks to sediment-dwelling organisms. Most chemicals with low water solubility sorb to soil particles before being transported into surface waters during heavy rainfall (Ghadiri & Rose, 1991). Runoff from treated lands thus has the potential to introduce new pulses of pesticide into aquatic systems through soil-adsorbed residues. Water-borne transport of pyrethroids from the farm site to major tributaries will occur largely in the particle-adsorbed form. Most information on pyrethroid toxicity is based on dissolved phase exposures, and there is little information on toxicity or dietary bioaccumulation resulting from ingestion of contaminated sediments or prey. We will address these limitations in the current state of knowledge. Our goal is to insure that CALFED's ecosystem restoration efforts are not compromised by pyrethroid-related impairment of water and sediment quality.

We will examine the following hypotheses:

1. Sediments are likely to serve as a long-term repository for pyrethroid residues. (Task 1)

The log K_{ow} of most pyrethroids is about 6 (range 4 to 7), comparable to the more hydrophobic polycyclic aromatic hydrocarbons (PAH) like benzo[a]pyrene or organochlorine pesticides like DDT. We expect rapid adsorption to both organic and inorganic suspended particles, and ultimately incorporation in sediments. While pyrethroids persist for only a short time in water (usually days to weeks), there is potential for greater persistence in bedded sediment; cypermethrin, fenvalerate, and permethrin have all been shown to persist in sediment for many months (Sharom & Solomon 1981 Schimmel et al. 1983, Giddings et al. 2001).

2. Particle-adsorbed pyrethroids are bioavailable, and ingestion of these particles will serve as a long-term source for contaminant uptake by aquatic biota. (Task 2)

Hydrophobic contaminants like PAH and PCB are largely accumulated via the diet (Landrum & Robbins 1989, Weston et al. 2000), and due to their hydrophobic character pyrethroids are likely to be accumulated the same way. Particle-bound contaminants are generally less bioavailable than those in the dissolved phase, however, when sedimentary ingestion occurs, dietary uptake of sediment-associated contaminants may be a major route of uptake. Bioavailability of pyrethroids to sediment-ingesting organisms is likely to be a function of sediment characteristics including organic carbon content and its composition

3. Pyrethroids tend to be rapidly biotransformed, but metabolic capabilities differ among taxa, and some taxa with poor biotransformation ability will pass toxicologically-active compounds on to their predators. (Tasks 3 and 4)

Little information is available on how fish and invertebrates process these chemicals (e.g. toxicokinetics) or on transfer of pyrethroids to higher trophic levels. Molluscs are relatively insensitive to pyrethroids, and have a high bioaccumulation potential. The bioconcentration factor (BCF) of fenvalerate in oysters is >4,700 (Clark et al. 1989), and in our laboratory experiments we have found esfenvalerate concentrations up to 22 μg/g in clams (*Macoma balthica*). The question arises whether species with a poor metabolizing capacity for pyrethroids may be able to transfer these toxicants through the food web to fish.

4. Chronic dietary uptake of toxicologically active pyrethroids by benthic-feeding fish causes sublethal effects which can be quantified by biomarker analyses. (Task 4)

Results of a pilot study (Werner et al. 2001) show that dietary uptake of the pyrethroid esfenvalerate leads to induction of certain stress proteins and may reduce reproductive success in the fish medaka. In addition, short-term exposure to esfenvalerate increases the incidence of histopathologic lesions in brains of Sacramento splittail (Teh et al. 2001). These subtle impacts are difficult to detect but may have considerable negative effects on fish populations. There is a need to understand and quantify the potential impacts of short-term and chronic exposure to pyrethroids on fish, in particular on reproductive success and individual organism health.

5. Rapid detection tools for pyrethroid exposure and/or effect in field collected organisms can be developed based on biomarker responses and specific metabolic enzymes. (Task 4).

The chemical analysis of pyrethroids in water samples at toxicologically relevant concentrations is difficult, costly, and not necessarily linked to biological availability. One of our objectives, the development of appropriate pyrethroid specific indicators of exposure, will focus on the activity of carboxylesterase, known to play a major role in pyrethroid detoxification in fish (Glickman et al.1981). In addition, we will develop rapid methods to analyze certain stress protein groups if they prove useful as indicators of cellular responses to pyrethroids.

6. Mixture effects among mulitple pyrethroids and with organochlorine insecticides having similar modes of action are toxicologically additive. (Task 5)

Current approaches to risk assessment generally assume additive effects for chemicals with the same mode of action. While these assumptions are valid for some mixtures, interactions between contaminants can be other than additive (Pape-Lindstrom & Lydy 1997, Belden & Lydy 2000). More information is needed on interactive effects of multiple pyrethroids and between pyrethroids and organochlorine insecticides that are similarly neurotoxic (e.g., DDT, DDE). Knowledge of synergistic or antagonistic interactions will improve risk assessments by providing important information on acceptable levels of pyrethroid insecticides in aquatic systems.

To address these hypotheses we propose a three-year study with (1) field work at farm sites, (2) a regional survey of pyrethroid sediment concentrations and (3) laboratory studies of bioaccumulation and toxicity. The work is divided into six tasks as described below.

3. Approach

TASK 1 - Field studies *(Primary responsibility: Weston, Participating: All P.I.s)*

Our field work will involve quantification of pyrethroids in soil, sediment and water samples. Expected analytical procedures are presented in Appendix 1. Based on literature review and our own analytical experience with pyrethroids, we are confident these procedures will achieve detection limits of 0.01-0.05 ppb in water and sediment and will be adequate for the work proposed. We refer to "pyrethroids" generically throughout this proposal, but the analytical approach will provide quantification of all members of the class currently in use in California agriculture (permethrin, esfenvalerate, cypermethrin, bifenthrin, cyfluthrin, and *lambda*-cyhalothrin).

In situ toxicity of runoff –*In situ* toxicity testing has several advantages over conventional laboratory-based tests. It captures the dynamic conditions of natural exposure that can not be replicated in the laboratory, but at the same time it retains experimental control. *In situ* testing is of particular value for pyrethroids because the strong surface adsorption characteristics of the class can result in adsorption to glassware and other surfaces associated with handling the sample, and the short aquatic half-life of many of the compounds can result in loss of toxicity in the time required to set up lab tests. While *in situ* toxicity testing has not been widely used in California, its merit has been demonstrated in many studies elsewhere (e.g., Chappie & Burton 1997, Ireland et al. 1996). It is an innovative approach that is well-suited to our proposed research goals.

Pyrethroid use as dormant sprays has been increasing in almond and stone fruit orchards, and because use coincides with winter rainfall that can result in runoff, we will focus our field studies on these industry segments. The specific farms we will examine will be identified early in the project in collaboration with the companion proposal from Zalom et al. The project teams will coordinate sampling efforts, and since both teams have extensive contacts throughout the agriculture industry, we have not had and do not anticipate any problems in identifying study sites, gaining access, and being kept informed of pesticide use. Both teams will also coordinate with other investigators who are working on off-site pesticide movement (e.g. Gary Obenauf of Agricultural Research Consulting, Sacramento River Watershed Group, Agricultural Implementation Group – San Joaquin Valley) to identify appropriate sites and obtain good grower cooperation. We are aware

of several orchard properties with adjacent streams, and some of these properties are located far up in the watershed with little or no upstream agricultural activity. This situation will allow deployment of *in situ* toxicity testing chambers above and below the farms under study, and an ability to link changes in toxicity or pyrethroid water concentrations to the types and amounts of pesticide used at that specific farm. There is minimal data in the literature on pyrethroid concentrations in water or sediment adjacent to treated lands, and such data during and after treatment would be extremely useful.

We intend to work at two sites both of which will apply pyrethroid pesticides (most likely permethrin or esfenvalerate) as dormant sprays, but differing in the potential for off-site migration of pesticide residues due to site-specific characteristics (e.g., ground cover, soil type). At each site we will conduct the studies described below, and studies will be repeated in the first and second winter of the project (potentially at different farms during second winter). Our goal is to determine the impact of pyrethroids entering surface water bodies via stormwater runoff. While toxicity of undiluted runoff within the orchard has been shown (Werner et al., in press(a)), these *in situ* tests will provide information on whether runoff into surface waters in the vicinity of treated orchards is toxic to aquatic life, and if toxicity persists in subsequent rainfall events long after treatment.

Field toxicity tests using caged macroinvertebrates and fish will be conducted at the farm sites at upstream, adjacent and downstream locations. Organisms will be exposed to the water column and to the sediments in separate exposure chambers designed to isolate these two environmental compartments. In order to verify that the organisms used for *in situ* testing are healthy, controls will be set up in the laboratory. Test organisms will include the same species used in laboratory studies discussed later and which vary significantly in anticipated pyrethroid sensitivity (laboratory cultured juvenile Sacramento splittail (*Pogonichthys macrolepidotus*), the midge, *Chironomus tentans*, the oligochaete, *Lumbriculus variegatus* and the cladoceran, *Ceriodaphnia dubia*). The use of *C. dubia* will allow direct comparison to laboratory toxicity tests of runoff using the same species by Zalom et al. *In situ* chambers consist of transparent core tubing (cellulose acetate butyrate) with two windows covered with polypropylene mesh (74 μm). Four replicate chambers will be deployed for each treatment (e.g., water column, sediments) and each species at each testing location. Test duration will range from 2-4 d, and survival will be determined. In addition, splittail will be analyzed for the pyrethroid-induced biomarker responses identified in Task 4 (see below). Water samples will be taken concurrently and analyzed for pesticides including pyrethroids. We anticipate doing *in situ* toxicity tests before and during pyrethroid spraying, after the first major rainfall event following spraying and following one or two rainfall events later in the season. We have had no difficulty in getting the grower cooperation needed to time these tests relative to pesticide application, and anticipate no problem obtaining cooperation in these studies.

Persistence of residues – Our work will focus on pyrethroid use during the dormant (i.e. winter) season. In previous work, Zalom et al. focused on either almond orchards (40% of which are not sprayed in-season) or stone fruit orchards where the grower agreed not to use in-season treatment of the pesticide of concern. We will use this same strategy. Winter-only treatment provides an opportunity to examine the persistence of residues in soils and sediments. Before treatment and afterwards (for up to 10 months) we will collect triplicate samples of soil and aquatic sediments in nearby watercourses. We will also obtain soils that accumulate in the runoff retention tanks of Zalom et al., thus quantifying residues on soil particles moving off-site. These samples will be analyzed for the pyrethroid(s) that had been applied and will tell us how long residues persist.

Regional assessment of pyrethroid concentrations in sediments – There are no available data on pyrethroid concentrations in the Bay/Delta watershed, but such data are needed to put toxicity

results gathered under Task 2 (below) in an environmentally relevant context. We will therefore survey sediments on two occasions during the project, once during the wet season shortly after application (i.e., late Feb. or early March) and again in the dry season (i.e. August to Sept.).

To gain a broad regional perspective 8 sites will be sampled in conjunction with the Department of Water Resources' benthic monitoring program in the Delta from northern San Francisco Bay up the Sacramento River to Rio Vista and up the San Joaquin River to Stockton. We will accompany DWR on two occasions and collect surface sediments from 8 of their 10 sampling sites for pyrethroid analysis (letter from DWR attached).

In addition to this broad geographical overview, we will sample locations that represent near-farm and near-urban conditions. We will sample surface sediments at 15 sites in highly agriculturally impacted watercourses (small sloughs, creeks or ditches adjacent to agricultural land) or in urban-affected waterbodies (e.g. Arcade Creek of Sacramento, Smith Canal of Stockton). At these sites we would expect pyrethroids, if present, to be at higher concentrations than in the larger sloughs and rivers that DWR samples. The precise location of the samples will be determined from land use and pesticide use practices. As part of our site identification effort we will coordinate with three demonstration projects of alternative practices to reduce off-site pesticide movement (CWA 319(h) grant to California Prune Board, Prop. 13 grant to California Almond Board, CALFED Watershed grant to Agric. Research Consulting). We may be able to locate some of our sites near their demonstration sites to help evaluate the success of their practices (letter of support attached).

We will also sample suspended sediment at 2-4 sites (e.g., Sacramento River at Greene's Landing and San Joaquin River at Vernalis) using a continuous flow centrifuge. Suspended sediment sampling will be during the wet season immediately after the first major rainfall after dormant spray use (typically late January). This suspended material is likely to be largely of recent terrestial origin and is expected to have the highest pyrethroid concentrations of all sediment samples. We will supplement these data with results from 9 other suspended sediment samples we are currently collecting and processing through a separate collaborative project with USGS.

These three sampling efforts (regional bedded sediment, near-farm/near-urban bedded sediment, and suspended sediment) should provide the first overview of pyrethroid sediment distributions. Comparison of the two seasonal data sets should provide some indication of seasonal persistence. These data will be invaluable in interpreting our own sediment toxicity data gathered in Task 2 and future data from the Delta and its watershed.

TASK 2 – Invertebrate toxicity and bioaccumulation ***(Primary responsibility: Weston)***

Particle-adsorbed pyrethroids introduced into aquatic systems have been shown to cause behavioral changes in a deposit-feeding fish, the gizzard shad (Drenner et al. 1993), to impact metabolism, growth and survival of a shrimp (McKenney 1998), and to reduce growth and emergence of aquatic insect larvae (Schulz & Liess 2001). Some of these studies found no pesticides in the dissolved phase during the exposures even with detection limits as low as 0.003 µg/L. From studies with other organic compounds with hydrophobicities comparable to those of the pyrethroids (e.g., Weston et al. 2000), it is clear that ingestion of contaminated particles can be a major, if not the dominant, route of uptake in deposit-feeding organisms.

Invertebrate toxicity – The first component of Task 2 will be to establish what levels of sediment contamination are acutely toxic to several sediment-associated invertebrates. There is very little information in the literature on this issue, yet, it is essential for an ecological interpretation of the pesticide concentration data gathered in Task 1 and in any future studies. The test species will represent a range of sediment-associated taxa: a midge (*Chironomus tentans*), a mayfly larvae (*Hexagenia* sp.), an amphipod (*Hyalella axteca*), an oligochaete (*Lumbriculus variegatus*), and a mollusc (*Corbicula fluminea*; will require sediment in suspension). Four of these species (*C.*

fluminea excluded) are routinely used for sediment quality testing, and several of these species (*C. tentans*, *H. azteca* and *C. fluminea*) are important constituents of the diet of fish species including salmon, splittail, and white sturgeon (Peterson 1997, Toft 2000, Feyrer 2000, Sommer et al. 2001).

Approximately 8 sediments of varying grain size and organic carbon content will be collected from streams and reservoirs. They will be obtained from the eastern Central Valley, upstream of the major dams and major agricultural areas in order to minimize concentrations of pyrethroids or other pesticides that could cause interactive effects in our studies. Low pesticide concentrations in these 8 test sediments will be analytically verified.

Test sediments will be spiked with ^{14}C-permethrin and ^{14}C-esfenvalerate (in separate treatments), the two most heavily used pyrethroids in California. Use of radiolabelled pesticides has several advantages. The cost per sample for quantification of pesticide residues by radioactivity is small, and the detection limit of radiolabelled compounds is lower than with conventional chemistry, allowing us to measure pyrethroids in the overlying water of our exposure systems and thus to quantify the extent of sediment desorption. Tests will generally run for10 days.

This experimental protocol will allow us to quantify the level of sediment contamination above which acute toxicity is noted and to determine LC_{50} values. We will also have data on dissolved concentrations due to desorption which we can compare with literature values to see if exposure via water contributes to toxicity. Sediment toxicity data will be invaluable in interpreting the field data on pyrethroid levels and will contribute to an understanding of the role of organic carbon in mitigating bioaccumulation (discussed below).

Invertebrate bioaccumulation – It is widely recognized that sediment contaminant concentrations are often poor predictors of bioaccumulation because sediment composition affects bioavailability. For other hydrophobic organic compounds, sediment organic carbon content is a key factor, and generally inversely proportional to bioavailability. We expect that the same will be the case for pyrethroids. Adsorption of *lambda*-cyhalothrin has been shown to be an order-of-magnitude greater on clay particles with an organic coating than on pure clay particles (Zhou et al. 1995).

Our objective is to understand the factors that influence pyrethroid bioavailability from sediment and help to quantify the risk of a given contaminant level. Among our test species, we expect *Lumbriculus variegatus* and *Corbicula fluminea* to be the most likely to accumulate substantial amounts of pyrethroids without toxic effects and thus to be potential vectors for transfer of active substances to higher trophic levels (Task 4). We will use *L. variegatus* for these bioaccumulation tests, and it is a standard species for such purposes (USEPA 2000). We will spike our 8 test sediments with ^{14}C-permethrin and ^{14}C-esfenvalerate (separate trials) at sublethal levels and determine bioaccumulation factors at steady state (time to reach steady state will be determined in preliminary experiments or from Task 3). The role of sediment organic content will be addressed by correlating BAF to natural organic carbon variation among the 8 sediments and possibly by further manipulating organic content using an external carbon source. The influence of particle-pesticide interaction time (i.e., "aging") on bioavailability will also be determined. Bioavailability of other hydrophobic compounds decreases with aging (Landrum 1989), and we expect that pyrethroid contaminated soils that enter aquatic systems in later rainfall events may be less toxic, even if equally contaminated, than soils washed into aquatic systems shortly after pesticide application.

Digestive fluid extraction - *In vitro* digestive fluid extraction is a recently developed technique that uses digestive fluid of a deposit-feeding invertebrate as an extractant for contaminated sediments instead of strong organic solvents used in traditional analytical procedures. The digestive fluid approach has far greater ecological relevance than organic solvents because it mimics the digestive processes occurring in an animal's gut that affect dietary solubilization and therefore bioaccumulation of sediment-adsorbed contaminants. The approach has shown promise (Weston &

Mayer 1998, Ahrens et al., 2000, Weston & Maruya, in press) to assess contaminant bioavailability from sediments without the lengthy and costly exposure period of whole animal bioaccumulation tests or the confounding influence of contaminant biotransformation (a significant issue with pyrethroids). Digestive fluid extraction has been used to measure bioavailability of PAH, PCB, hexachlorobenzene, and a wide variety of metals. We are currently doing work with USGS and UC funding to extend the approach to particle-adsorbed pesticides. With funding from the U.S. Army Corps of Engineers, we are developing a synthetic mimic of natural digestive fluid that could be used for dredged material assessment.

Digestive fluid extractions will be done in parallel with the bioaccumulation tests discussed above, and the same questions addressed with the oligochaete bioaccumulation tests (e.g., influence of organic carbon, sediment aging) will be assessed by digestive fluid extraction. Both natural digestive fluid from the polychaete *Arenicola brasiliensis* and the synthetic version will be evaluated. Our goal is to determine if the amount of contaminant desorbed from sediment by digestive fluid extraction is a good predictor of the risk that sediment poses to a benthic organism in terms of contaminant bioaccumulation. The digestive fluid approach has been shown to predict risk for other compounds (Weston & Maruya, in press), and if it is equally useful for pyrethroids, then scientists and resource managers will have a powerful new tool for use in the Delta and tributaries.

TASK 3 – Toxicokinetics ***(Primary responsibility: Lydy)***

Toxicokinetics is the study of the rate processes involved in uptake, distribution, metabolism and elimination of a toxic chemical in an organism. This information is critical when judging the potential for toxicity and bioconcentration of chemicals. This component of the project will determine toxicokinetic parameters such as uptake clearance coefficients (k_u), elimination rate constants for both parent compound (k_{ep}) and metabolites (k_{em}), the biotransformation rate (k_m), biological half-life ($t_{1/2}$) and bioconcentration factors (BCF) for each compound. In addition, toxicokinetic models will be developed for two pyrethroids in four species (*Chironomus tentans*, *Lumbriculus variegatus*, *Corbicula fluminea* and *Pogonichthys macrolepidotus*) which are selected for phylogenetic diversity, for their anticipated differing abilities to metabolize pyrethroids, and because toxicokinetic information on these species are needed for other project tasks.

The toxicokinetics work will be conducted in water only exposures for splittails (no sediment), while sediment exposures will be used for the invertebrates. Uptake clearance of each compound from the dosed matrix will be measured by placing animals into water or sediment spiked with a radiolabelled pyrethroid (either ^{14}C-permethrin or ^{14}C-esfenvalerate). Specific sampling times will be determined in preliminary studies for each of the test species, but a general plan is to collect samples at 1, 2, 4, 6, 12, 24, 48, 72 and 96 hr for the splittail tests and 0.5, 1, 2, 4, 6, 8, 10 days for the sediment exposures. At each sampling time, levels of radioactivity in animals, water and sediment will be analyzed. Biotransformation potential will be determined by fractionating tissue activity into parent or metabolites by thin layer chromatography (TLC) or by high performance liquid chromatography (HPLC) using the methods of Lydy et al. (2000). We will also conduct longer-term (16 wk) toxicokinetic studies with splittail in connection with Task 4.

Elimination rate constants will be measured by placing contaminated test organisms (exposed as above) into uncontaminated water or sediment with the overlying water being completely replaced 3-4 times per day. Triplicate samples of organisms will be withdrawn at each sampling time. Duration and frequency of sampling will both depend on elimination rate.

Toxicokinetic parameters will then be determined using a two-compartment model to describe the distribution of compounds in the chosen species (Lydy et al. 2000). This model incorporates uptake from the environment, rate of biotransformation from parent compound to metabolites and elimination rates for both the parent compound and metabolites (see Figure 1). Data

will be collected so that the uptake and elimination phases can be modeled simultaneously. An iterative least squares fit of the data will be done using the following differential equations:

$$\frac{dC_{tot}}{dt} = (k_u C_w) - (k_{ep} C_p) - (k_{em} C_m) \qquad \frac{dC_p}{dt} = (k_u C_w) - (k_m C_p) - (k_{ep} C_p) \qquad \frac{dC_m}{dt} = (k_m C_p) - (k_{em} C_m)$$

where:, C_p, C_m, and C_{tot} = conc. of parent compound, metabolites and their total, respectively, in animal; C_w = conc. of chemical in water; k_u = uptake clearance coefficient; k_{ep} and k_{em} = parent and metabolite elimination rate constants; k_m = biotransformation rate constant; and t = time. This equation, based on water exposures, will be slightly modified for species exposed via sediment. Bioconcentration factors, or bioaccumulation factors in the case of sediment exposure, will be estimated from the kinetics using the following equation:

$$BCF = \frac{C_p}{C_w} = \frac{k_u}{(k_{ep} + k_m)}$$

To properly evaluate a BCF for a metabolized compound, both the elimination rate of parent compound (k_{ep}) and loss rate via biotransformation (k_m) must be considered. The biological half-lives of parent compound ($t_{1/2p}$) and metabolites ($t_{1/2m}$) will also be determined.

The toxicokinetic data will be invaluable in interpreting the bioaccumulation results for fish and invertebrates (Tasks 2 and 4). The results will also identify which taxa have minimal biotransformation abilities and thus will be most likely to pass toxic residues on to predators (Task 4).

TASK 4 – Fish toxicity *(Primary responsibility: Werner, Participating: Teh, Gee)*

Conventional wisdom is that pyrethroids have little potential to bioaccumulate through the food chain because of rapid metabolism (Hill 1985). However, aquatic organisms tend to metabolize pyrethroids much slower than warm-blooded terrestrial organisms. Even fish, which might be expected to metabolize them rapidly, will retain parent compound for several days (Coats et al. 1989). Biotransformation capabilities are even weaker in some invertebrate taxa such as molluscs and some annelids, and we suspect persistence of pyrethroids in their tissues may provide a route for uptake of residues by their predators.

In a pilot study with the fish medaka (Werner et al., in press(b)), we found that chronic dietary exposure of fish to pyrethroids can cause adverse sublethal effects. A diet containing esfenvalerate at 148 mg/kg did not cause mortality after 7 days, but resulted in sublethal effects such as reduced reproductive ability and induction of stress proteins. Considering the high bioconcentration factor of pyrethroids in some mollusks (>4700; Clark et al. 1989), such concentrations in the diet of benthic feeding fish like Sacramento splittail may well be environmentally realistic. In addition, given half-lives of many months for these compounds in sediments (Section 2 above) fish can be chronically exposed for long periods.

We will study exposure and potential deleterious effects of pyrethroids on Sacramento splittail, a species that is threatened and particularly appropriate to this study because of its benthic foraging habits (Feyrer, 2000). We will measure physiological and reproductive effects, and the expression pattern of biomarkers such as certain stress proteins, lysosomal membrane integrity, histopathological lesions and the activity of pyrethroid metabolizing enzymes. Increased expression of stress proteins is indicative of the activation of the cellular protein repair system (e.g., Werner & Nagel 1997, Sanders 1993). Specifically, we propose to investigate if the induction of hsp60 and hsp90 proteins observed in our pilot project is indicative of a specific response pattern useful to detect pyrethroid effects in field situations. Both stress protein groups are associated with cellular receptor function. A reduction in lysosomal integrity indicates sublethal cellular damage and signals a reduced ability to maintain normal cellular function (e.g. Koehler 1991, Giamberini & Pihan 1997). Histopathologic biomarkers are lesions in cells, tissues, or organs caused by exposure to toxic agents, and histopathologic damage in reproductive organs can be directly linked to

reproductive health (Wester & Canton 1991). Organ-specific lesions can also be tied to specific modes of action (Teh et al. 2001). Esterase and P450 are key enzymes in pyrethroid metabolism and their activity indicative of the level of exposure and susceptibility to toxicity.

Dietary exposures - Initially, we will establish short-term toxicity thresholds for individual pyrethroids through 14-day dietary exposure. Sacramento splittail will be obtained from a colony maintained at UC Davis by one of our investigators (Teh). Currently, there are 1000 splittail available for experimental study, and 5000 embryos and larvae will become available during the spawning season each year. Fish will be fed their standard casein-based diet (controls) or modified to include the pesticide. We will test a range of concentrations to determine LD_{50} or ED_{50} for each compound. Endpoints measured will be mortality, growth and biomarker responses (see below).

Long-term (4-month) dietary exposures, most likely using esfenvalerate, will be based on information obtained through the field studies, invertebrate bioaccumulation and toxicokinetic studies (Tasks 1, 2 and 3). Oligochaetes and *C. fluminea*, two of our invertebrate test species, occur naturally in the diet of splittail (Meng & Moyle 1995, Feyrer 2000) and we will mimic pyrethroid concentrations likely to be encountered in these species based on results of the previous tasks. A diet containing the pyrethroid will be prepared by mixing the pesticide with the normal dry ingredients via an emulsion made from a mixture of methanol and corn oil. In addition to regular control diet, a methanol-corn oil diet will be used as a solvent control. Additionally we will do a shorter term and smaller scale study using fish diets containing pyrethroid-contaminated invertebrate tissue using *L. variegatus* or *C. fluminea* reared on contaminated sediments, following Task 2 procedures. Dietary exposure with contaminated tissue vs. feed spiked with pure compound will allow us to assess bioavailability and better evaluate the risk of exposure via the natural diet. Fish will be fed a ration of 10% body weight/day in two feedings (morning, afternoon). After the 4-month exposure period, a subset of the fish from each treatment will be sexed by gonad biopsies and maintained through spawning and hatching (approx. 8 months) to measure effects on reproductive success.

Biological effects measurements - Survival rates will be quantified, and growth will be measured by weighing fish before and after the exposures. We will take samples after 4 months of exposure and just before spawning to measure the following endpoints.

Physiological indices: Gross measurements and weights will be used to determine condition index (CI), gonadosomatic (GSI) and hepatosomatic (HSI) indices in the fish. CI is a measure of "plumpness" and defined as body weight/ length x100. GSI is the gonad to body weight ratio and HSI is the liver to body weight ratio. All three indices are broad measures of general health. Changes in CI specifically reflect alterations in growth and nutritional status, while fluctuations in GSI are associated with sexual maturity and reproductive status. Differences in HSI may reflect sex, sexual maturity, or general health and nutritional status. Gonadosomatic and hepatosomatic indices have proven to be sensitive and simple indicators of responses when comparing fishes from contaminated and reference sites (Jobling et al. 1996).

Histopathological indicators: Histopathology will be the primary means of assessing contaminant-related adverse effects in organs and tissues of fish. It will also be used to determine effects of endocrine disruption (intersex, aresia of oocytes, necrosis of spermatogonia). Tissues for routine histopathology will include: liver, kidney gill, gonad, and brain. Samples for histopathology will be fixed in 10% neutral buffered formalin and processed (dehydration, infiltration, and embedding) in paraffin. Paraffin blocks will be sectioned at 4-6 µm, mounted on glass slides, and stained with hematoxylin and eosin. All lesions will be semi-quantitatively scored (0 = not present, 1 = mild, 2 = moderate, 3 = severe) based on size and number.

Lysosomal membrane integrity: Serial cryostat sections (10 µm) of the liver will be incubated according to Koehler (1991) for different time periods to determine the lysosomal destabilization time. Lysosomal destabilization time, the time of acid labilization needed to destabilize the membrane, is marked by the maximum staining intensity of acid phosphatase in lysosomes and will be assessed by image analysis.
Stress protein analysis: Hsp proteins (hsp60, hsp70, hsp90) will be analyzed in liver, muscle, gonads and gills using Western blotting techniques. Monoclonal antibodies for hsp70 and hsp90 (1:500; Affinity Bioreagents, StressGen) and a polyclonal antibody for hsp60 (1:1000; StressGen) will be used as probes, and bound antibody will be visualized with a chemiluminescent substrate (CDP-Star; Tropix, Bedford, MA) and quantified by densitometry.
P450/EROD activity: A fluorometric method that measures activity of the enzyme ethoxyresorufin O-deethylase (EROD) will be used to quantify CYP1A1 (Munkitterick et al.,1995) in liver. EROD activity is catalyzed by CYP1A1 (P450), and increases when CYP1A1 expression is induced. Tissue samples are homogenized in Tris homogenization buffer, and centrifuged at 10000 x g for 20 min. The supernatant is collected and centrifuged at 100000 x g for 1hr. The resulting microsomal pellet is resuspended. Samples are loaded into microplate wells, and 20µL ethoxyresorufin solution are added to each well. Resorufin fluorescence is measured on a microplate reader at 530 nm (excitation) and 585 nm (emission).
Reproductive effects: We will determine fecundity by histological screening of the ripe adult gonads of 10 fish just before spawning occurs. After spawning of the remaining fish, subsets of 100 embryos per treatment group will be reared to quantify hatching success and larval viability.

Development of rapid monitoring assays for pyrethroid exposure and effect - Esterases and P450s are known to metabolize pyrethroids in fish (Glickman et al. 1981). Long term, low level exposure to pyrethroids may affect enzyme levels and this change may be useful as a biomarker of exposure and effect. In order to provide a rapid tool for assessment of pyrethroid exposure, we intend to develop a selective assay for monitoring levels of esterases associated with pyrethroid hydrolysis. We also propose to develop a novel substrate to screen for fenvalerate-selective esterases based on the use of fluorogenic substrates. Commercial porcine esterases will be used in initial substrate screening and evaluation. Following initial screening, esterases from control and exposed splittail will be evaluated for catalytic activity using liver samples. Kinetic assays will be run using a microplate reader using absorbance or fluorescence modes as appropriate. All activities will be corrected for background hydrolysis and specific activities normalized for protein concentration using methods of Bradford (1976). To probe esterase diversity, we will use several esterase substrates that we have synthesized in our laboratory (Huang et al. 1996), including two highly sensitive, novel fluorogenic esterase substrates recently developed by Shan and Hammock (2001).

If stress protein or enzymatic activities prove to be useful indicators of pyrethroid exposure and/or effect, methods will be developed to facilitate rapid measurement of these parameters. For example, the same antibodies that are used for western blotting analysis of stress proteins may be formatted into a rapid 96-well plate immunoassay based on technology we already have available.

TASK 5 - Pesticide mixtures ***(Primary responsibility: Lydy, Werner)***

Our understanding of interactions of pesticides in mixtures is generally limited and restricts our ability to predict impacts of environmental contamination. Little if any research has been conducted examining interactions among pyrethroid insecticides or between pyrethroids and organochlorine pesticides that are also neurotoxins with modes of action similar to pyrethroids. The objective of this section is to investigate, for selected species, potential interactions among pyrethroids and among pyrethroids and organochlorines, and classify these interactions as additive, synergistic or antagonistic.

Mixture toxicity testing will be performed on two species, the midge *C. tentans* and larval Sacramento splittail, and will be determined using a modified toxic unit approach (Pape-Lindstrom & Lydy 1997). In the toxic unit (TU) model, a value of 1 TU is assigned to the LC_{50} (or LD_{50}if internal dose) value of each contaminant. A sum of the TU contributed by each component describes the toxicity of a mixture as follows:

$$TU_{sum} = \frac{C_{w_1}}{LC_{50_1}} + \frac{C_{w_2}}{LC_{50_2}} + ... + \frac{C_{w_i}}{LC_{50_i}}$$

where: Cw_i is the concentration of a chemical in a mixture and LC_{50i} is the LC_{50} for the respective component chemicals of the mixture from 1 to i. Empirically measured toxicity can then be compared to expected toxicity which is generated using LC_{50} values determined in tests of individual toxicants. When 50% mortality occurs at TU values lower than 1, the mixture is exhibiting greater than additive toxicity (synergism). Determination of less than additive toxicity (antagonism) is made when 50% mortality occurs at TU values greater than 1.

We will initially establish a LC_{50} for individual pesticides in solution (bifenthrin, esfenvalerate, cypermethrin, permethrin, *lamda*-cyhalothrin, DDT, DDE). Acute toxicity testing will be conducted in static systems for 96 h. The LC_1, LC_5, LC_{15} and LC_{50} values will be determined for each pesticide using probit analysis. Acute toxicity tests with binary mixtures will be conducted in a manner similar to the individual pesticide tests. Concentrations of each pesticide will be added at proportions of their respective LC_{50} so that the sum of concentrations of the pesticides is equivalent to five concentrations: 0.5 TU, 0.75 TU, 1.0 TU, 1.5 TU, and 2.0 TU. Actual mortality in mixture tests will be compared to predicted toxicity assuming additive effects.

In addition to these mixture studies using aqueous exposures, dietary mixture studies will be done with splittail using the same suite of pyrethroids and organochlorines. Short-term dietary exposures (14 days) will be conducted according to the methods described under Task 4 and above (aqueous mixture studies). We will use LD_{50}s determined under Task 4 to test potential additivity of binary mixtures equivalent to 0.5, 0.75, 1, 1.5 and 2 TU. Endpoints measured will be mortality, growth and biomarker responses as described in Task 4. Long-term dietary mixture studies (4 months of exposure followed by 8 months until spawing) will be performed using environmentally realistic concentrations of selected pyrethroid and DDT/DDE. Endpoints measured will be mortality, growth, endocrine and reproductive effects and biomarker responses.

TASK 6 - Project Management ***(Primary responsibility: Weston, Participating: All P.I.s)***

Project Management has been identified as a distinct task as requested by the PSP. Tasks include preparation of quarterly, annual and final reports and the QA/QC plan, participation in project coordination meetings, review of co-investigators' products, oral presentations of results to CALFED or in similar forums, etc.

4. Feasibility

Measuring pyrethroids in the dissolved phase and using these data in conjunction with studies of bioaccumulation or toxicity presents challenges because present analytical techniques are unable to detect pyrethroids at concentrations lethal to the most sensitive species. A current SWRCB contract with UC Davis and a current proposal to CALFED (Kuivila et al.) both seek to improve analytical techniques, but adequate techniques do not currently exist.

Recognizing these analytical limitations, we have structured our study to minimize or avoid them. First, we are emphasizing sediments as a source for chronic exposure. Given the hydrophobicity of pyrethroids, concentrations on sediments are orders-of-magnitude greater than in water and thus more readily quantified. Our field studies necessarily incorporate some water sampling near points of pesticide use (adjacent streams where concentrations are likely to be

highest), but most of our analytical effort is directed towards sediments. Secondly, much of our laboratory work utilizes radiolabelled compounds. Rather than quantifying pyrethroid concentration by conventional analytical techniques, we will use radioactivity, with sensitivity greater than possible by conventional means and with lower cost/sample. Finally, we have included Shirley Gee on our project team. She is the principal investigator on the SWRCB contract with UC Davis to develop new toxicity testing procedures for pyrethroids, and will help make available any advances.

A second key attribute of our project is the link with the proposal of Zalom et al. to develop effective but environmentally protective approaches for managing target pests that have historically been controlled with OP insecticides. Their approaches include evaluating non-OP pest controls for efficacy and nontarget species effects, improved application technologies to lower pesticide amounts applied and reduce off-target movement, and best management practices to reduce off-site movement of dormant spray pesticides. We have included Frank Zalom on our project team to insure coordination between the two groups in field sampling, to keep us aware of pesticide use practices, help in identification of field sites, and promote access to farms and grower cooperation. To the maximum extent possible, our field work will utilize the same farm sites as Zalom et al., and we will time our field studies to focus on the same pesticide use events. We will also use the runoff studies of Zalom et al. in interpreting our *in situ* toxicity tests. We hope that CALFED will fund both projects to maximize the synergistic value of the two studies, but we can accomplish our goals independently. Our study can continue with minor modification to portions of the Task 1 field component should the Zalom et al. project not be funded.

Finally, this project incorporates two relatively new techniques in ecotoxicology. Using digestive fluid extraction to measure contaminants that may be bioavailable to deposit feeders was first proposed by Mayer et al. (1996), and since then approximately 12 papers on the approach have appeared in peer-reviewed literature. The extraction technique is intuitively attractive because of its obvious ecological relevance, and it has proven value in measuring bioavailability of organic compounds of similar hydrophobicities to pyrethroids (Weston & Mayer 1998, Ahrens et al. 2000). *In situ* toxicity testing has been underutilized in the Central Valley, although it has been extensively used in freshwater streams of the central U.S. It has inherently greater environmental realism than standard laboratory toxicity tests, and eliminates concerns such as handling losses including glassware adsorption of the toxicologically active agents.

5. Performance measures

We suggest the following be used as performance measures for this research project:

Presentations

Metric: Number presentations given to stakeholders and/or in scientific conferences.

Target: We anticipate that 2 presentations will be given in each year of the project and that a total of at least 6 will be given over the three years of the study.

Newsletters

Metric: Number of articles appearing with substantial coverage of this research.

Target: We anticipate at least 2 newsletter articles over the duration of the project.

Publications

Metric: Number of peer-reviewed publications

Target: We anticipate a minimum of five peer-reviewed publications arising from this work. In addition, at least one technical report (final report to CALFED) will be provided.

Final research product

Metric: Correlation of digestive fluid extraction technique with in vivo bioaccumulation

Target: By project completion we anticipate demonstrating the utility of the digestive fluid extraction technique in assessing bioavailability of pesticides and will promote its use by resource agencies or other parties involved in water or sediment toxicity testing.

Final research product
Metric: Demonstration of the *in situ* technique for toxicity testing.
Target: By project completion we anticipate demonstrating the utility of the *in situ* toxicity testing procedure and its adoption by resource agencies or other parties involved in toxicity testing.
Final research product
Metric: Availability of rapid monitoring tools for the detection of pyrethroid exposure and effect
Target: Sensitive and selective assays for biological and physiological studies following pyrethroid exposure will be developed based on biomarker results. The objective is to provide rapid, toxicologically relevant tools to detect exposure and effects of pyrethroids in aquatic systems.

6. Data handling and storage

Most of the data collected will be summarized using standard statistical methods (e.g. calculation of means and standard deviations). To test for significant differences, data meeting assumptions of normality and homogeneity of variance will be subjected to analysis of variance (ANOVA), followed by posthoc comparisons using Dunnett's test or Student-Newman-Keuls (SNK) test. In cases where parametric assumptions are not met, Kruskal-Wallis or the Wilcoxon two-sample test will be used to test for differences among treatments and controls.

Data for each task will be maintained by the investigator responsible for the task, most commonly in Excel worksheets. Data will be regularly backed-up and archived. Finalized data will be available for review upon request, and summary data will be made available through regional newsletters (e.g., IEP), reports to CALFED and peer-reviewed publications.

7. Expected products/outcomes

This research will provide environmental management authorities with information needed to assess risks posed by particle-associated pesticides, and if necessary, to take steps to protect aquatic species and habitats. Currently, no one is looking for pyrethroid residues in sediments, and even if they were, the information does not exist to determine if any given level represents a risk to biota. This study will provide essential information including: 1) concentrations of pyrethroids in a variety of habitats; 2) levels of sediment contamination at which acute toxicity is observed; 3) factors influencing bioavailability of sediment-associated pesticides; 4) ability of various species to metabolize these pesticides (with ramifications for these species and for the potential for trophic transport); 5) potential for toxicological interactions with organochlorines already in aquatic sediments; and 6) potential effects of dietary exposure to pyrethroids for threatened fish species. Finally, we will provide new tools for managing environmental quality in the Delta and its watershed including *in vitro* digestive fluid extraction, *in situ* toxicity testing, and rapid enzyme-based techniques for exposure assessment.

In addition to a completion report to CALFED, we anticipate this work will result in a minimum of five publications in peer-reviewed literature. We also anticipate one or more articles in newsletters (e.g., IEP newsletter) and several presentations in various forums (e.g. Society of Toxicology, Society of Environmental Toxicology and Chemistry). To facilitate incorporation of our findings in management strategies we will reach regional environmental managers through regional newsletters (IEP newsletter) and oral or poster presentations (State of the Estuary Conference, CALFED Science Conference, Sacramento River Watershed Program, etc.)

8. Work schedule

For the purpose of this proposal we have assumed a July 1, 2002 start date. A three-year project is proposed with tasks to be completed as shown in Figure 2. Field work will primarily be during winter months of 2002/2003 and 2003/2004, but sampling for environmental persistence of pesticide residues will continue for approximately 10 months after pesticide use. Laboratory tasks

Figure 2. Anticipated project schedule.

	Project yr 1								Project yr 2									Project yr 3					
	2002								2003									2004					
	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J
Task 1 (Field studies)							X	X	X	X	X	X	X	X	X	X	X	X	X	X				
Task 2 (Invert. tox./bioaccum)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Task 3 (Toxicokinetics)		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Task 4 (Fish toxicity)				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Task 5 (Mixtures)																		X	X	X	X	X	X	X
Task 6 (Project management)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

will all run concurrently and continue through much of the project period. A final report will be provided at the end of Year 3.

The PSP asks that proposals address the feasibility of partial funding. We have carefully designed the proposed tasks to be complementary and to address interrelated aspects of the larger question of environmental effects of pyrethroids. We obviously feel that each task makes a unique contribution to knowledge of pyrethroid fate and effects and that the paucity of information currently available clearly calls for a substantial, multifaceted research effort. However, if only partial funding were made available, funding of either Tasks 2 or 4 would require funding of the related portion of Task 3 (either invertebrates or fish toxicokinetics) for proper data interpretation.

B. APPLICABILITY TO ERP, SCIENCE PROGRAM, IMPLEMENTATION PLAN, AND CVPIA

1. ERP, Science Program and CVPIA priorities

The proposed project is most directly applicable to ERP Goal 6: "Sediment and Water Quality" as the output of the work will be assessment of sediment and water quality and the threats posed to aquatic life. The work also contributes to ERP Goal 1: "At-risk species" and ERP Goal 3: "Harvestable species" because of its emphasis on splittail and benthic invertebrates that are of important trophic value to salmon (chironomid prey) or sturgeon (bivalve prey). The work meets many goals of the CVPIA because we will be studying pyrethroid toxicity to invertebrate species (espec. chironomids) which are prominent in the salmon diet.

This research fits well within the CALFED Science Program and its priorities of adaptive management, enhanced interdisciplinary knowledge of critical unknowns, and improvement in the scientific basis of water management. Management of the environmental effects of pyrethroids is not currently possible since little is known about them in general and virtually nothing is known about pyrethroids in habitats of this watershed. The broad database we will gather will allow environmental authorities to begin adaptively managing effects of pyrethroids which are of emerging importance.

The proposed work fits under many priority areas addressed by the PSP. It is explicitly identified under two priority topics:

Multi-regional priority 5: Ensure that restoration is not threatened by degraded environmental quality - Within this priority is a stated interest in understanding exposure to and effects of pyrethroid pesticides.

Delta Region priority 6: Restore shallow water habitats in the Delta for the benefit of at-risk species while minimizing potential adverse effects of contaminants – Within this priority is a stated need for fate and effects data on pyrethroids in eastside tributaries, floodplains, inundated Delta islands and tidal wetlands.

In addition, with its consideration of splittail, important salmon prey species, and/or promoting a greater understanding of contaminant impacts, the work also fits under SR-7, SJ-3, SJ-5, and BR-5.

2. Relationship to other ecosystem restoration projects

We currently have funding through CALFED (99-N08) to study effects of pesticides on invertebrates of trophic importance to juvenile salmon. This on-going study was initiated before increasing pyrethroid use was generally recognized and therefore focuses primarily on OP pesticides, especially chlorpyrifos. We are not sampling sediments, where pyrethroids are more likely to be found. As pyrethroid use has increased, we have attempted to modify the current project to some degree to accommodate some research on pyrethroids. We have, for example, planned some studies of pulse dosing with pyrethroids and some biomarker development work. Pyrethroids, however, are a small component of our current project, and our ongoing work will help to produce preliminary data that will allow us to better design the studies planned in this proposal.

Though they are not funded through the Ecosystem Restoration Program, there are three other projects with which we will coordinate. Demonstration projects for alternative practices to reduce off-site pesticide movement are currently being funded by a CWA 319(h) grant to the California Prune Board, a Prop. 13 grant to the California Almond Board, and a CALFED Watershed grant to Agricultural Research Consulting. We will work with the project manager for these efforts (Gary Obenauf; letter of collaboration attached) in identifying our study sites.

We are aware of three other proposals being submitted under the 2002 PSP with which we could coordinate. First, there is our intended collaboration with Zalom et al. that has been previously discussed. Secondly, a proposal by SFEI et al. will examine toxicity of unknown cause and attempt to identify causes in both water and sediment samples, primarily by TIE methods. No TIE methods, however, have been developed for pyrethroids, and toxicity due to this group of compounds could prove difficult to verify. Our results, particularly those involving sediment exposures with invertebrates (Task 2) could provide data of considerable value to the SFEI et al. research group. Thirdly, Kuivila et al. have submitted a proposal to improve analytical methods for pyrethroids. We are interested in their proposed work, and if it is successful, we will incorporate their methodologies in our field studies. However, we recognize the current analytical limitations for measuring pyrethroids in water and thus have chosen to emphasize sediments in our field work and to use radiolabelled compounds in our laboratory work to avoid these analytical difficulties.

3. Requests for next-phase funding – NOT APPLICABLE

4. Previous recipients of CALFED or CVPIA funding

a. CALFED 99-N08 (Assessment of pesticide effects on fish and their food resources in the Sacramento-San Joaquin Delta): Weston, Werner and Lydy currently have funding to study the effect of pesticides on chinook salmon and on invertebrates of trophic importance to juvenile chinook salmon. We completed a major field effort in the winter of 2000/2001 in which we saw little water column toxicity (sediment not studied) in the Sacramento and San Joaquin Rivers and generally low concentrations of OPs. We have developed new toxicity tests with a resident species and are progressing well on studies of herbicide/OP mixture toxicity and realistic pesticide exposure scenarios (instead of typical single exposure 96 hr tests). This study is currently about half completed. In the event the current proposal is funded, there will be only a 9-month overlap between the two projects.

b. CALFED 97-C12 (Alternative practices for reducing pesticide impacts on water quality): Zalom and Werner are in the final year of a project to evaluate and promote alternatives to OP pesticides. The project has produced information matrices for urban and agricultural pesticide applicators to evaluate alternatives, produced on-line and other educational material, refined procedures for indigenous species toxicity testing, and provided a wealth of data on pesticides in orchard runoff that is serving as a basis for further mitigation work. A final report has been submitted to CALFED. In the event the current proposal is funded, there will be little overlap in time and none in content between the two projects

c. CALFED 99-N07 (Chronic Toxicity of Environmental Contaminants in Sacramento Splittail (*Pogonichthys macrolepidotus)*: A Biomarker Approach: Teh and collaborators have completed two seasons of field sampling and three laboratory studies. Two papers have been submitted to peer-review journals. 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. There is no overlap between the two projects.

5. <u>System-wide ecosystem benefits</u>

This research has both direct and indirect potential benefits to at-risk species identified by CALFED. The proposed work directly addresses toxicity of pyrethroid pesticides to Sacramento splittail. This species is among those to be used in the toxicokinetic studies; it will also be used to study the effects of chronic dietary exposure to pyrethroids on metabolic indicators of stress and the incidence of histopathological disorders which have clear population level ramifications. Fish, along with arthropods, tend to be the most sensitive organisms to pyrethroid toxicity, thus we believe it important to include a fish species in our studies, and particularly one as important as the splittail.

Our research could also indirectly benefit salmon or demersal-feeding fish such as sturgeon that consume the invertebrate species we will be investigating. *Chironomus tentans* will be used in many study components, and we have found chironomids to be the dominant prey organism of juvenile fall-run chinook as they move seaward through the lower Sacramento River from January to April (unpub. data recently collected under CALFED 99-N08). Chironomids are of equal importance as prey in the upper Sacramento and tributary streams (Moore 1997). As the salmon move into the Delta, amphipods, particularly *Hyalella azteca,* become increasingly important in their diet (Toft 2000), and we will study this amphipod as well. Understanding and mitigating the effects of pyrethroids on invertebrates is of critical importance not only to protecting these species, but also at-risk salmon and other fish species that depend upon them for food.

In vitro digestive fluid extraction has not been applied to water quality issues in the Central Valley and we believe *in situ* toxicity testing has not been used to its full potential. The testing and application of these new approaches that will be accomplished through this project will illustrate the potential of these techniques, make regional investigators aware of them, and lead to their broader application to a host of environmental issues through the Bay/Delta watershed. While our study focuses on pyrethroids, these techniques have utility in studies of other pesticides, mercury, selenium, and other contaminants.

C. QUALIFICATIONS

Dr. Donald Weston is an Associate Adjunct Professor in the Dept. of Integrative Biology, University of California, Berkeley. He has approximately 20 years experience in studying the effects of anthropogenic contaminants on benthic invertebrates, both at the individual and community level. His research emphasizes issues pertaining to the bioavailability of sediment-associated contaminants, and he has directed many studies involving toxicity testing with aquatic invertebrates. He has17 peer-reviewed publications on the bioaccumulation and/or toxicity of pollutants to aquatic invertebrates. He will be lead investigator on the proposed project and will also have primary responsibility for much of the field work and the invertebrate toxicity and bioaccumulation components.

Ms. Shirley Gee is a Staff Research Associate in the Department of Entomology at UC Davis. She has published more than 12 peer-reviewed articles in the area of comparative metabolism and has been a leader in the development of pesticide immunoassay as an analytical method for environmental and human exposure monitoring for the past 15 years resulting more than 40 publications. This expertise will be utilized in this project toward the development of a biomarker of effect of pyrethroid exposure based on carboxylesterase activity.

Dr. Michael Lydy is an environmental toxicologist at Southern Illinois University. He has been conducting research on toxicokinetics, toxicity and bioavailability of pesticides in aquatic systems for 16 years. Because of his extensive experience working with pesticide mixtures and analytical method development, he has been included in this project as lead investigator for the toxicokinetics and pesticide analyses, and a collaborator on the mixture task. He also will play a role in the development and implementation of the *in situ* toxicity bioassays.

Dr. Swee Teh is a comparative pathologist with 14 years of extensive field and laboratory research experience in ecotoxicology and biomarker studies. He will be primarily responsible for the histopathological and histochemical assessment of in situ and laboratory exposed fish, and will closely coordinate with Dr. Werner on the fish bioaccumulation and pesticide mixture studies. He has been Principle Investigator on and managed grants from various Federal agencies, including USEPA, NCI, and CALFED. Dr. Teh is an author on over two dozen peer-reviewed publications related to invertebrate and fish histopathology, histochemistry, and ecotoxicology.

Dr. Ingeborg Werner of UC Davis, School of Veterinary Medicine will be responsible for coordination of tasks among the other UCD project participants, and will share the technical responsibilities in fish *in situ* exposures (Task 1), toxicity (Task 4) and mixture experiments (Task 5). Dr. Werner has 10 years of experience in biomarker research and aquatic toxicity testing. 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 organization.

Dr. Frank G. Zalom is an entomologist in the Agricultural Experiment Station and Cooperative Extension Specialist at UC Davis. He has studied integrated pest management (IPM) for California fruit and nut crops for 21 years and served as Director of the University of California Statewide IPM Program for 16 years. He has published 150 journal articles and book chapters and interacts widely with growers, pest control advisers and others associated with the agricultural industry. Dr. Zalom is a Fellow of the California Academy of Sciences, President of the Entomological Society of America - Pacific Branch, and Chair of the National IPM Committee of the National Association of State Universities and Land Grant Colleges. He will provide agricultural practices input to this project, and coordination with the companion CALFED proposal.

D. COST

1. Budget (submitted as web form)

2. Cost-sharing

The total cost share committed to this project is $373,368, consisting of two components. First, the U.S. Army Corps of Engineers (ACOE) is currently funding UC Berkeley to develop a synthetic digestive fluid that can be used to measure bioavailability of sediment-associated contaminants. This fluid will be available for use in Task 2 of the proposed project. Therefore the anticipated $333,368 value of years 2 and 3 of the ACOE project (the period when the fluid will be developed) is offered as a match providing the CALFED award is made from state funds (federal funds not eligible as match for federal award). Award of year 3 funding from ACOE is dependent upon Congressional appropriation in fall of 2002, but we fully expect its award.

Secondly, matching funds totaling $40,000 will be provided by Southern Illinois University in the form of funds for a new proportional diluter and water polishing (purification) systems for Dr. Lydy's laboratory to allow him to conduct the planned experiments.

E. LOCAL INVOLVEMENT

Our field work (e.g., soil persistence, *in situ* toxicity testing) will involve sampling on or adjacent to private land. Permission from the growers before sampling and their active collaboration will be necessary (i.e., informing us of impending pyrethroid use and modifying pesticide use to achieve project objectives). In a previously funded CALFED project (97-C12) we have obtained good grower cooperation in these areas and anticipate the same in the proposed work. We have extensive contacts with individual growers, pest control advisers, and with agricultural industry groups (e.g., Almond Board of California, California Prune Board, Coalition for Urban/Rural Environmental Stewardship, Almond Pest Management Alliance) as well as county Cooperative Extension offices that will facilitate local involvement.

We also anticipate involving local groups with interest in water quality issues by frequent oral presentations of project results. Among the groups to which we anticipate giving presentations are the Sacramento River Watershed Program, and its various committees (Monitoring, Toxics), Agricultural Implementation Group - San Joaquin Valley, and California Agricultural Production Consultants Association (CAPCA).

F. COMPLIANCE WITH STANDARD TERMS AND CONDITIONS

The project applicant will take exception to the following: "Rights in Data, Acknowledgements, and Peer Review" provision in Chapter 4.2 of the Proposal Solicitation, and standard clauses from Attachment D of the ERP Proposal Solicitation Package: Section 2 (Payment Schedule), Section 3 (Performance Retention), Section 6 (Substitution), Section 9 (Rights in Data), Section 11 (Indemnification), and Section 13 (Termination Clause).

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Appendix 1. Planned analytical procedures for pyrethroid analyses

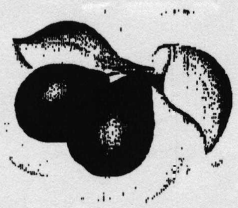
Water procedures - Four-L water samples will be collected for pesticide analysis. Samples will be stored in amber glass bottles for transportation to the lab and subsequent extraction via solid phase extraction (SPE) techniques. Supelco C_{18} columns will be preconditioned by pre-eluting with 3 ml of hexane:acetone 1:1, 3 ml methanol, and 5 ml reagent water. The sample will be subsequently extracted by passing 1 to 4-L of water through the column at 20 psi (10-18 ml/min.). Sample bottles will also be rinsed with hexane to ensure no loss of pesticide due to sorption to the glassware. The column will be allowed to air dry for five minutes, and then the analytes will be extracted from the column using three 5 ml rinses of 1:1 hexane: acetone. All extracts will be evaporated to 1 ml under a gentle stream of nitrogen prior to analysis by gas chromatography (GC).

GC analysis of the extracts will be performed on a Hewlett Packard 6890 Gas Chromatograph equipped with an electron capture detector and a split-splitless injector (220 °C, split-less, 0.75 min. purge time). The Supelco capillary column will be either a DB-608 or DB-5, 30 m x 0.320 mm with a 0.50 μm film thickness. Oven temperatures and gas flow rates will be determined from preliminary experiments. Qualitative identification will be based upon retention times within 0.50 % of standards, while quantitation will be based upon peak area utilizing external standards.

Sediment/Soil/Tissue procedures - The extraction method for the sediment/soil/tissue is a modification of USEPA Method #3550 (Sonication extraction for low concentrations of organics and pesticides). Thirty grams of media (sediment, soil or tissue) will be mixed with anhydrous sodium sulfate and extracted using 100 ml of 50:50 methylene chloride:acetone (v/v). The sample will be sonicated for three minutes (Tekmar Sonic Disruptor fitted with a Model CV26 Sonicator, output control set at 10, pulse mode), decanted, and filtered through a Whatman No. 41 filter paper filled with anhydrous sodium sulfate. This procedure will be repeated three times, each with an additional 50 ml of solvent. The extract will then be collected in an evaporative flask and reduced to approximately 10 ml, under vacuum, using a RE111 Rotavapor and a Büchi 461 Water Bath. After cooling, the extract will be solvent exchanged with hexane and the volume further reduced to 5 ml, under nitrogen gas, using a Pierce Model 1878 Reactivap. Cu^{2+} will be used to remove residual sulfur from the sediment samples, while florisil or GPC cleanup of the samples will be conducted following methods outlined in Sabaliunas et al. (1998). The concentrated extract will be transferred to clean screw-cap vials, sealed with a Teflon-lined lid, and stored in the dark at 4°C until analysis on GC as described above.

Total Organic Carbon and Lipid Determinations – Percent lipid in tissues and total organic carbon (TOC) in sediment and soils will also be determined. Lipids will be extracted from tissue samples using a solution of 50:50 methylene chloride:acetone (v/v). Each sample will be sonicated for 30 s, filtered through a Whatman No. 41 filter paper, thoroughly rinsed with solvent and dried at 50☜C overnight. The resulting lipid will be determined gravimetrically. TOC analysis will be performed using the methods of Harkey et al. (1994).

Quality assurance/quality control - QA/QC will include dual-column confirmation (DB608™ and DB5™), an extraction blank, and a blank spike (sediment samples) for each extraction batch. In addition, a surrogate recovery standard tetrachloro-m-xylene (TCMX) or decachlorobiphenyl (DCB) will be added to all samples prior to extraction. Each daily run or sequence will include a solvent blank and four calibration standards. A calibration verification standard will be run every 10 samples to insure that the calibration curve is within 15 % of the calibration range.



CALIFORNIA DRIED PLUM BOARD
3841 North Freeway Blvd. Phone (916) 565 6232
Suite 120 Fax (916) 565 6237
Sacramento, CA 95834 www.CaliforniaDriedPlums.org

September 18, 2001

Dr. Frank G. Zalom
Statewide IPM Project
One Shields Ave.
University of California
Davis, CA 95616

Dr. Donald Weston
University of California
1301 S. 46th St., Bldg. 112
Richmond, CA 94804

Dear Drs. Zalom and Weston:

I am very interested in the studies you are proposing to the CALFED Ecosystem Restoration Program entitled "Water Quality Effects of Pesticides used in Orchard Agriculture - Part 1: Evaluating Management Alternatives and Off-site Movement" and "Part 2: Aquatic Fate and Effects of Particle-sorbed Pyrethroids". I am currently managing several studies to develop Best Management Practices for pesticide use in orchards through a CWA 319(h) grant, a Prop. 13 grant, and a CALFED Watershed grant, and am eager to explore ways in which we could coordinate our efforts. I would be happy to help identify farms where conditions are best suited for your study requirements. In addition, I understand you would be sampling aquatic sediments for pesticide residues, and perhaps I could help identify sample locations that both meet your needs and assist in evaluation of our BMP work. I strongly support your proposed work, and I would be happy to assist in any way I can.

Sincerely,

Gary L. Obenauf

Gary L. Obenauf

STATE OF CALIFORNIA -- THE RESOURCES AGENCY GRAY DAVIS, *Governor*

DEPARTMENT OF WATER RESOURCES
ENVIRONMENTAL SERVICES OFFICE
3251 S STREET
SACRAMENTO, CA 95816-7017

September 20, 2001

Dr. Don Weston
UC Berkeley
Richmond Field Station
1301 S. 46th Street Building 112
Richmond California 94804.

Dear Dr. Weston:

I have great interest in your proposed CALFED study on "Water Quality Effects of Pesticides used in Orchard Agriculture – Part 2: Aquatic Fate and Effects of Particle-sorbed Pyrethroids". The Monitoring and Analysis Branch of the Department of Water Resources has monitored water quality and aquatic organisms in the Sacramento-San Joaquin Delta for nearly 30 years as part of the Interagency Ecological Program, Environmental Monitoring Program. While our focus is on detecting the effects of water project operations on water quality and biota, we also have to consider other factors such as pesticide toxicity to correctly interpret our monitoring data. As pyrethroid use becomes more and more common, a better understanding of its effects is urgently needed for effective management of our estuarine resources.

DWR currently monitors benthic organisms and sediment particle composition on a monthly basis at ten sites from San Pablo Bay to the southern and northern Delta. As pyrethroid pesticides may have their greatest effect on benthic organisms, we are very interested in coordinating our sampling efforts with you and assisting with the field aspect of your study. Specifically, we are willing to contribute sediment sampling assistance and boat access during our monthly benthic monitoring runs.

We strongly support this proposal to investigate pyrethroid effects in the Delta and are looking forward to collaborating with you on this project.

Sincerely,

Z. P. Hymanson

Zachary Hymanson, Chief
Monitoring and Analysis Branch

DEPARTMENT OF THE ARMY
ENGINEER RESEARCH AND DEVELOPMENT CENTER, CORPS OF ENGINEERS
ENVIRONMENTAL LABORATORY
WATERWAYS EXPERIMENT STATION, 3909 HALLS FERRY ROAD
VICKSBURG, MISSISSIPPI 39180-6199

REPLY TO
ATTENTION OF:

September 20, 2001

CEERD-EP-R

Mr. Don Weston
UC Berkeley
Integrative Biology
1301 S. 46th St.
Richmond, CA 94804

Dear Don,

Thank you for informing me of your intention to submit a proposal to the CALFED Ecosystem Restoration Program entitled "Water Quality Effects of Pesticides Used in Orchard Agriculture - Part 2: Aquatic Fate and Effects of Particle-sorbed Pyrethroids". I understand one component of this work involves using digestive fluids of deposit-feeding invertebrates or a synthetic extractant designed to mimic those fluids as a means to measure pyrethroid bioavailability. The Army Corps of Engineers (ACOE) is currently supporting your research on synthetic digestive fluids through a Broad Agency Agreement (BAA) with UC Berkeley, and the techniques developed through this Agreement should be directly applicable to your proposed CALFED work. Therefore it would be appropriate to consider our funding to UC Berkeley through the BAA as leverage/cost share in the CALFED proposal.

Year 1 under the BAA is near complete, and it is my expectation that we will continue to fund this work for 2 additional years. Since the research emphasis in years 2 and 3 will be toward development of the synthetic digestive fluid, the value of the contract in these two years may be the most appropriate to consider as leverage for the CALFED work. Award of year 2 funds ($166,684) is imminent, and if year 3 is presumed to have a comparable value, then the total eligible leverage would be $333,368. The actual value of the award in years 2 and 3 would depend upon Congressional appropriation and programmatic allocation of funds within ACOE, but the value provided above is my expectation and best estimate.

The ACOE is supporting development of digestive fluid extraction techniques because we believe they will prove helpful in evaluation of dredged material, but we are eager to see other applications such as that proposed to CALFED. I hope our work will assist with your proposal and demonstrate the potential merit that we see in the technique for sediment risk assessment.

Sincerely,

Todd S. Bridges, Ph.D.
Research Biologist