

# **Identifying The Causes Of Feminization Of Chinook Salmon In The Sacramento And San Joaquin River System**

prepared by Sedlak, David L

submitted to Science Program 2004

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# Project

This proposal is for the Science Program 2004 solicitation as prepared by Sedlak, David L.

The submission deadline for this proposal has passed. Proposals may not be changed.

## Instructions

Information provided on this form will automatically support subsequent forms to be completed as part of the Science PSP submission process. Please be mindful of what information you enter and how it may be represented in the Personnel, Task and Budget forms. Please provide this information before continuing to those forms.

**Proposal Title** *Identifying the Causes of Feminization of Chinook Salmon in the Sacramento and San Joaquin River System*

**Institutions** University of California, Berkeley  
University of California, Riverside  
Applied Marine Sciences  
Southern Nevada Water Authority

*List each institution involved, one per line.*

**Proposal Document**

*You have already uploaded a proposal document. [View it](#) to verify that it appears as you expect. You may replace it by uploading another document*

**Project Duration** *36 months*

Is the start date a determining factor to the successful outcome of the proposed effort?

No.

Yes. Anticipated start date of this effort:

Select all of the following study topics which apply to this proposal.

- life cycle models and population biology of key species
- environmental influences on key species and ecosystems
- relative stresses on key fish species
- direct and indirect effects of diversions on at-risk species
- processes controlling Delta water quality
- implications of future change on regional hydrology, water operations, and environmental processes
- water management models for prediction, optimization, and strategic assessments
- assessment and monitoring
- salmonid-related projects
- Delta smelt-related projects

Select as many keywords as necessary to describe this proposal (minimum of 3).

- adaptive management*
- aquatic plants*
- benthic invertebrates*
- biological indicators*
- birds*
- neotropical migratory birds
- shorebirds
- upland birds
- wading birds
- waterfowl
- climate*
- climate change
- precipitation
- sea level rise
- snowmelt
- contaminants / toxicants / pollutants*
- contaminants and toxicity of unknown origin
- emerging contaminants
- mercury
- nutrients and oxygen depleting substances
- organic carbon and disinfection byproduct precursors
- persistent organic contaminants

- X pesticides
  - salinity
  - sediment and turbidity
  - selenium
  - trace metals
  - **database management**
  - **economics**
  - **engineering**
  - civil
  - environmental
  - hydraulic
  - **environmental education**
  - **environmental impact analysis**
  - **environmental laws and regulations**
  - **environmental risk assessment**
  - **fish biology**
  - bass and other centarchids
  - delta smelt
  - longfin smelt
  - other species
- X salmon and steelhead
  - splittail
  - striped bass
  - sturgeon
  - **fish management and facilities**
  - hatcheries
  - ladders and passage
  - screens
  - **forestry**
  - **genetics**
  - **geochemistry**
  - **geographic information systems (GIS)**
  - **geology**
  - **geomorphology**
  - **groundwater**
  - **habitat**
  - benthos
  - channels and sloughs
  - flooded islands
  - floodplains and bypasses
  - oceanic
  - reservoirs
  - riparian
  - rivers and streams
  - shallow water
  - upland habitat
  - vernal pools
  - water column
  - wetlands, freshwater
  - wetlands, seasonal
  - wetlands, tidal
  - **human health**
  - **hydrodynamics**
  - **hydrology**
  - **insects**
  - **invasive species / non–native species / exotic species**
  - **land use management, planning, and zoning**
  - **limnology**
  - **mammals**
  - large
  - small
  - **microbiology / bacteriology**
  - **modeling**
  - conceptual
  - quantitative

- *monitoring*
- *natural resource management*
- *performance measures*
- *phytoplankton*
- *plants*
- *primary productivity*
- *reptiles*
- *restoration ecology*
- *riparian ecology*
- *sediment*
- *soil science*
- *statistics*
- *subsidence*
- *trophic dynamics and food webs*
- *water operations*
- barriers
- diversions / pumps / intakes / exports
- gates
- levees
- reservoirs
- *water quality management*
- ag runoff
- mine waste assessment and remediation
- remediation
- temperature
- urban runoff
- X water quality assessment and monitoring
- *water resource management*
- *water supply*
- demand
- environmental water account
- water level
- water storage
- *watershed management*
- *weed science*
- *wildlife*
- ecology
- management
- wildlife-friendly agriculture
- *zooplankton*
- *administrative*

Indicate whether your project area is local, regional, or system-wide. If it is local, provide a central ZIP Code. If it is regional, provide the central ZIP Code and choose the counties affected. If it is system-wide, describe the area using information such as water bodies, river miles, and road intersections.

- local	ZIP Code:
- regional	ZIP Code: counties:
X system-wide	Sacramento and San Joaquin River between freshwater delta and upstream salmon spawning areas

Does your project fall on or adjacent to tribal lands?

*No.*

*(Refer to California Indian reservations to locate tribal lands.)*

If it does, list the tribal lands.

Has a proposal for this effort or a similar effort ever been submitted to CALFED for funding or to any other public agency for funding?

*No.*

If yes, complete the table below.

**Status Proposal Title Funding Source Amount Comments**

Has the lead scientist or principal investigator of this effort ever submitted a proposal to CALFED for funding or to any other public agency for funding?  
*No.*

If yes, provide the name of the project, when it was submitted, and to which agency and funding mechanism if was submitted. Also describe the outcome and any other pertinent details describing the proposal's current status.

All applicants must identify all sources of funding other than the funds requested through this solicitation to support the effort outlined in their proposal. Applicants must include the status of these commitments (tentative, approved, received), the source, and any cost-sharing requirements. Successful proposals that demonstrate multiple sources of funding must have the commitment of the non-Science Program PSP related funding within 30 days of notification of approval of Science Program PSP funds. If an applicant fails to secure the non-Science Program PSP funds identified in the proposal, and as a result has insufficient funds to complete the project, CBDA retains the option to amend or terminate the award. The California Bay-Delta Authority reserves the right to audit grantees.

**Status Proposal Title Funding Source Period Of Commitment Requirements And Comments**

Are you specifically seeking non-federal cost-share funds for this proposal?  
*No.*

In addition to the general funds available, are you targeting additional funds set aside specifically for collaborative proposals?  
*Yes.*

List people you feel are qualified to act as scientific reviewers for this proposal and are not associated with CALFED.

Full Name	Organization	Telephone	E-Mail	Expertise
<i>Gary Ankley, Ph.D.</i>	<i>US EPA, Mid-Continent Ecology Division</i>	<i>218-720-5603</i>	<i>ankley.gerald@epamail.epa.gov</i>	<i>contaminants / toxicants / pollutants</i>
<i>Peter Matthiessen, Ph.D.</i>	<i>Centre for Ecology and Hydrology, Lancaster Environment Centre.</i>	<i>(UK) 01524-595867</i>	<i>pmatt@ceh.ac.uk</i>	<i>contaminants / toxicants / pollutants, emerging contaminants</i>
<i>Marc Suter, Ph.D.</i>	<i>EAWAG</i>	<i>(CH) 01823-5479</i>	<i>marc.suter@eawag.ch</i>	<i>contaminants / toxicants / pollutants, contaminants and toxicity of unknown origin</i>

**Executive Summary**

Provide a brief but complete summary description of the proposed project; its geographic location; project objective; approach to implement the proposal; hypotheses being tested; expected outcomes; and relationship to Science Program priorities. The Executive Summary should be a concise, informative, stand-alone description of the proposed project. *(This information will be made public on our website shortly after the closing date of this PSP.)*

Results of a recent study indicate that up to 38% of the male Chinook salmon in the Sacramento and San Joaquin Rivers are feminized. It is believed that the feminization of salmon is attributable to one or more chemical contaminants that alter sexual differentiation of larval salmon. Several chemical contaminants that are present in surface waters where larval salmon reside, including steroid hormones from livestock and detergent metabolites associated with pesticide applications, can cause feminization of fish at concentrations comparable to those expected in surface waters. The purpose of this project is to identify the agents responsible for feminization of salmon in the Sacramento and San Joaquin Rivers. To achieve this objective, chemical analyses and bioassays will be used to analyze samples collected between the freshwater delta and the upstream spawning areas. Samples exhibiting biological activity will be subjected to chemical fractionation and exhaustive chemical analyses to identify the causative agents. Results of the research will be used to identify cost-effective approaches for controlling or preventing feminization of salmon and other important fish species. This research project is relevant to CALFED's efforts to protect and restore Chinook salmon and other critical species because it provides much needed information about a family of chemical stressors that have not received much attention from the CALFED program.

Give additional comments, information, etc. here.

# Applicant

This proposal is for the Science Program 2004 solicitation as prepared by Sedlak, David L.

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All information on this page is to be provided for the agency or institution to whom funds for this proposal would be awarded.

**Applicant Institution** *University of California, Berkeley*

*This list comes from the project form.*

**Applicant Institution Type** *public institution of higher education*

### Institution Contact

Please provide information for the primary person responsible for oversight of grant operation, management, and reporting requirements.

**Salutation** *Dr.*

**First Name** *David*

**Last Name** *Sedlak*

**Street Address** *657 Davis Hall*

**City** *Berkeley*

**State Or Province** *CA*

**ZIP Code Or Mailing Code** *94720*

**Telephone** *510 643 0256*  
*Include area code.*

**E-Mail** *sedlak@ce.berkeley.edu*

Additional information regarding prior applications submitted to CALFED by the applicant organization or agency and/or funds received from CALFED programs by applicant organization or agency may be required.

## Personnel

This proposal is for the Science Program 2004 solicitation as prepared by Sedlak, David L.

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## Instructions

Applicants must provide brief biographical sketches, titles, affiliations, and descriptions of roles, relevant to this effort, of the principal and supporting project participants by completing a Personnel Form. This includes the use of any consultants, subcontractors and/or vendors; provide information on this form for all such people.

Information provided on this form will automatically support subsequent forms to be completed as part of the Science PSP submission process. Please be mindful of what information you enter and how it may be represented in the Task and Budget forms.

Information regarding anticipated subcontractor services must be provided regardless if the specific service provider has been selected or not. If the specific subcontractor has not been identified or selected, please list TBD (to be determined) in the Full Name field and the anticipated service type in the Title field (example: Hydrology Expert).

Please provide this information before continuing to those forms.

### Sedlak, David L., Ph.D.

*This person is the **Lead Investigator**. Contact information for this person is required.*

<b>Full Name</b>	<i>Sedlak, David L., Ph.D.</i>	example: Wright, Jeffrey R., PhD.
<b>Institution</b>	<i>University of California, Berkeley</i>	<i>This list comes from the project form.</i>
<b>Title</b>	<i>Professor</i>	<i>example: Dean of Engineering</i>
<b>Position Classification</b>	<i>primary staff</i>	
<b>Responsibilities</b>	Coordination of project implementation. Supervision of activities related to chemical analyses.	
<b>Qualifications</b>		<i>You have already uploaded a PDF file for this question. Review the file to verify that appears correctly.</i>
<b>Mailing Address</b>	<i>Department of Civil and Environmental Engineering, 657 Davis Hall</i>	
<b>City</b>	<i>Berkeley</i>	
<b>State</b>	<i>CA</i>	
<b>ZIP</b>	<i>94720</i>	
<b>Business Phone</b>	<i>510-643-0256</i>	
<b>Mobile Phone</b>	<i>510-643-0256</i>	
<b>E-Mail</b>	<i>sedlak@ce.berkeley.edu</i>	

Describe other staff below. If you run out of spaces, submit your updates and return to this form.

### Schlenk, Daniel, Ph.D.

<b>Full Name</b>	<i>Schlenk, Daniel, Ph.D.</i>	example: Wright, Jeffrey R., PhD. Leave blank if name not known.
<b>Institution</b>	<i>University of California, Riverside</i>	<i>This list comes from the project form.</i>
<b>Title</b>	<i>Professor</i>	<i>example: Dean of Engineering</i>
<b>Position Classification</b>	<i>primary staff</i>	
<b>Responsibilities</b>	Supervision of activities related to bioassays. Design of experimental activities and data interpretation.	

Qualifications		<i>This is only required for primary staff.</i>  <i>You have already uploaded a PDF file for this question. <u>Review the file</u> to verify that appears correctly.</i>
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### Kolodziej, Edward P., PhD.

Full Name	<i>Kolodziej, Edward P., PhD.</i>	example: Wright, Jeffrey R., PhD.  Leave blank if name not known.
Institution	<i>University of California, Berkeley</i>	<i>This list comes from the project form.</i>
Title	<i>Post-Doctoral Researcher</i>	<i>example: Dean of Engineering</i>
Position Classification	<i>primary staff</i>	
Responsibilities	Project management and project coordination; Chemical analysis, including analyte measurement, data interpretation, and data presentation, for both monitoring and toxicity identification evaluation tasks.	
Qualifications		<i>This is only required for primary staff.</i>  <i>You have already uploaded a PDF file for this question. <u>Review the file</u> to verify that appears correctly.</i>

### Spies, Robert B., PhD.

Full Name	<i>Spies, Robert B., PhD.</i>	example: Wright, Jeffrey R., PhD.  Leave blank if name not known.
Institution	<i>Applied Marine Sciences</i>	<i>This list comes from the project form.</i>
Title	<i>President</i>	<i>example: Dean of Engineering</i>
Position Classification	<i>primary staff</i>	
Responsibilities	Supervision and coordination of sample collection; implementation of choriogenin bioassay.	
Qualifications		<i>This is only required for primary staff.</i>  <i>You have already uploaded a PDF file for this question. <u>Review the file</u> to verify that appears correctly.</i>

### Post-Doctoral Researcher

Full Name		example: Wright, Jeffrey R., PhD.  Leave blank if name not known.
Institution	<i>University of California, Riverside</i>	<i>This list comes from the project form.</i>
Title	<i>Post-Doctoral Researcher</i>	<i>example: Dean of Engineering</i>
Position Classification	<i>secondary staff</i>	
Responsibilities	Development and implementation of in-vivo salmonid bioassays, YES assays, and toxicology aspects of the toxicity identification evaluation (TIE).	
Qualifications		<i>This is only required for primary staff.</i>  <i>Upload a <u>PDF version</u> of this person's resume that is no more than five pages long. To upload a resume, use the "Browse" button to select the PDF file containing the resume.</i>

## Graduate Student

<b>Full Name</b>		example: Wright, Jeffrey R., PhD. Leave blank if name not known.
<b>Institution</b>	<i>University of California, Berkeley</i>	<i>This list comes from the project form.</i>
<b>Title</b>	<i>Graduate Student</i>	<i>example: Dean of Engineering</i>
<b>Position Classification</b>	<i>secondary staff</i>	
<b>Responsibilities</b>	Assists with field sampling and chemical analysis, including experimental design, analytical chemistry, and data interpretation.	
<b>Qualifications</b>		<i>This is only required for primary staff.</i>  <i>Upload a <u>PDF version</u> of this person's resume that is no more than five pages long. To upload a resume, use the "Browse" button to select the PDF file containing the resume.</i>

## Subcontractor For Sampling Services

<b>Full Name</b>		example: Wright, Jeffrey R., PhD. Leave blank if name not known.
<b>Institution</b>	<i>Applied Marine Sciences</i>	<i>This list comes from the project form.</i>
<b>Title</b>	<i>Subcontractor for Sampling Services</i>	<i>example: Dean of Engineering</i>
<b>Position Classification</b>	<i>subcontractor</i>	
<b>Responsibilities</b>	Sample collection and shipping	
<b>Qualifications</b>		<i>This is only required for primary staff.</i>  <i>Upload a <u>PDF version</u> of this person's resume that is no more than five pages long. To upload a resume, use the "Browse" button to select the PDF file containing the resume.</i>

## Snyder, Shane A., Ph.D.

<b>Full Name</b>	<i>Snyder, Shane A., Ph.D.</i>	example: Wright, Jeffrey R., PhD. Leave blank if name not known.
<b>Institution</b>	<i>Southern Nevada Water Authority</i>	<i>This list comes from the project form.</i>
<b>Title</b>	<i>Research and Development Project Manager</i>	<i>example: Dean of Engineering</i>
<b>Position Classification</b>	<i>subcontractor</i>	
<b>Responsibilities</b>	Analysis of extracts by HPLC/MS/MS	
<b>Qualifications</b>		<i>This is only required for primary staff.</i>  <i>You have already uploaded a PDF file for this question. <u>Review the file</u> to verify that appears correctly.</i>

# Conflict Of Interest

This proposal is for the Science Program 2004 solicitation as prepared by Sedlak, David L.

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## Instructions

To help Science Program staff manage potential conflicts of interest in the review and selection process, we need some information about who will directly benefit if your proposal is funded. We need to know of 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.

**Applicant** University of California, Berkeley

**Submitter** Sedlak, David L

**Primary Staff** Sedlak, David L., Ph.D.

**Primary Staff** Schlenk, Daniel, Ph.D.

**Primary Staff** Kolodziej, Edward P., PhD.

**Primary Staff** Spies, Robert B., PhD.

**Secondary Staff** \*Post-Doctoral Researcher

**Secondary Staff** \*Graduate Student

**Subcontractor** \*Subcontractor for Sampling Services

**Subcontractor** Snyder, Shane A., Ph.D.

Are there other persons not listed above who helped with proposal development?

*No.*

If there are, provide below the list of names and organizations of all individuals not listed in the proposal who helped with proposal development along with any comments.

# Tasks

This proposal is for the Science Program 2004 solicitation as prepared by Sedlak, David L.

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## Instructions

Utilize this Task Table to delineate the tasks identified in your project description. Each task and subtask must have a number, title, brief description of the task (detailed information should be provided in the project description), timeline, list of personnel or subcontractors providing services on each specific task, and list of anticipated deliverables (where appropriate). When creating subtasks, information must be provided in a way that avoids dual presentation of supporting tasks within the overall task (i.e. avoid double counting). Information provided in the Task Table will be used to support the Budget Form. Ensuring information regarding deliverables, personnel and costs associated with subtasks are only provided once is imperative for purposes of avoiding double counting of efforts within the Budget Form.

For proposals involving multiple institutions (including subcontractors), the table must clearly state which institutions are performing which tasks and subtasks.

Task ID	Task Name	Start Month	End Month	Personnel Involved	Description	Deliverables
1.1	<i>Quantifying the occurrence of (xeno)estrogens using chemical analysis</i>	1	18	<i>Sedlak, David L., Ph.D. Kolodziej, Edward P., Ph.D. *Graduate Student *Subcontractor for Sampling Services</i>	Verify analytical methods (months 1–2). Collect and analyze water samples. Extract water samples and analyze extracts for (xeno)estrogens.	1) Measurements of (xeno)estrogen concentrations in over 200 water samples from the Sacramento and San Joaquin watersheds. 2) Analysis of data correlating spatial occurrence of these compounds with pesticide application rates and hydrologic conditions.
1.2	<i>Quantifying estrogen agonists in water samples with an in vitro bioassay</i>	1	18	<i>Schlenk, Daniel, Ph.D. *Post-Doctoral Researcher</i>	Analyze estrogen agonists in sample extracts from task 1.1 using an in vitro bioassay	1) Quantification of total estrogenicity in over 200 water samples. 2) Comparison of total estrogenicity with concentrations of (xeno)estrogens (Task 1.1) to identify locations where unknown (xeno)estrogens are present.
1.3	<i>Development of in vivo bioassays for feminization of early life-stage salmon</i>	1	18	<i>Schlenk, Daniel, Ph.D. *Post-Doctoral Researcher</i>	Test dose/response relationship for early-life stage salmon by imposing fish to different concentrations of compounds that have been shown to cause feminization of other, closely related fish species.	1) Developed and validated Chinook salmon bioassay, including dose-response relationships for (xeno)estrogens in water and sediments.
2.1	<i>Targeted sampling at selected locations</i>	18	36	<i>Sedlak, David L., Ph.D. Kolodziej, Edward P., Ph.D. *Graduate Student *Subcontractor for Sampling Services</i>	Collect samples from selected locations in task 1.1 where elevated concentrations of (xeno)estrogens were detected as well as additional nearby locations. Extract samples and analyze for chemical contaminants, YES activity and in vivo activity.	1) Quantification of (xeno)estrogens in over 50 water samples from locations identified in Task 1.1. 2) Quantification of (xeno)estrogens in approximately 100 water samples collected from watersheds with elevated (xeno)estrogen concentrations. 3) Identification of specific (xeno)estrogen sources in selected locations in the Sacramento and San Joaquin watersheds. 4) Description of specific strategies to control, reduce, and alleviate stressors related to (xeno)estrogens.
2.2	<i>Toxicity identification evaluation</i>	18	36	<i>Sedlak, David L., Ph.D. Schlenk, Daniel, Ph.D. Kolodziej, Edward</i>	Perform chemical fractionation studies coupled to bioassays on samples from Task 2.1 in which (xeno)estrogens are detected. Analyze fractions by chemical methods, in vitro and in vivo	1) Identification of unknown (xeno)estrogens, including likely sources and control strategies.

				<i>P., Ph.D. *Post-Doctoral Researcher *Graduate Student Snyder, Shane A., Ph.D.</i>	bioassays.	
2.3	<i>Conduct in vivo bioassays on selected samples</i>	18	36	<i>Schlenk, Daniel, Ph.D. *Post-Doctoral Researcher</i>	Perform in vivo bioassays with early life-stage Chinook salmon on samples from task 2.1 and 2.2 in which activity is detected.	1) Verification that (xeno)estrogens are responsible for observations of feminized Chinook salmon in the Sacramento and San Joaquin watersheds.
2.4	<i>Analyze extent of feminization with other fish species</i>	18	36	<i>Spies, Robert B., Ph.D. *Subcontractor for Sampling Services</i>	Collect fish plasma samples and analyze for choriogenins.	1) Evaluation of the extent to which other fish species in the watershed are feminized.

# Budget

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## Instructions

All applicants must complete a budget for each task and subtask. The Budget Form uses data entered in the Task Form, thus tasks should be entered before starting this form. Failure to complete a Budget Form for each task and/or subtask will result in removal of the application from consideration for funding.

CBDA retains the right to request additional information pertaining to the items, rates, and justification of the information presented in the Budget Form(s).

Supporting details on how costs were derived for each line item must be included in the justification section for each item. The cost detail for each item should include the individual cost calculations associated with each line item to provide the basis for determining the total amount for each budget category.

Following are guidelines for completing the justification section of this form:

### *Labor (Salary & Wages)*

Ensure each employee and associated classification is correctly identified for each task and subtask. This information will automatically be provided once the Staff Form has been completed. Provide estimated hours and hourly rate of compensation for each position proposed in the project.

### *Employee Benefits*

Benefits, calculated as a percentage of salaries, are contributions made by the applicant for sick leave, retirement, insurance, etc. Provide the overall benefit rate and specify benefits included in this rate for each employee classification proposed in the project.

### *Travel*

Travel includes the cost of transportation, subsistence, and other associated costs incurred by personnel during the term of the project. Provide purpose and estimated costs for all travel. Reoccurring travel costs for a particular task or subtask may be combined into one entry. The number of trips and cost for each occurrence must be clearly represented in the justification section for reoccurring travel items of this nature.

Any reimbursement for necessary travel and per diem shall be at rates specified by the California Department of Personnel Administration for similar employees ([www.dpa.ca.gov/jobinfo/statetravel.shtml](http://www.dpa.ca.gov/jobinfo/statetravel.shtml)).

### *Equipment*

Equipment is classified as any item of \$5,000 or more and has an expected life of three years or more. Equipment purchased in whole or in part with these grant funds must be itemized. List each piece of equipment and provide a brief description and justification for each.

### *Supplies*

Provide a basic description and cost for expendable research supplies. Costs associated with GIS services, air photos, reports, etc. must be listed separately and have a clear justification associated with each entry. Postage, copying, phone, fax and other basic operational costs associated with each task and subtask may be combined unless the cost associated with one particular service is unusually excessive.

### *Subcontractor Services*

Subcontractor services (Professional and Consultant services) include the total costs for any services needed by the applicant to complete the project tasks. Ensure the correct organization is entered in the Personnel Form so that it appropriately appears on the Budget Form. The applicant must provide all associated costs of all subcontractors (i.e. outside service providers) when completing this form. Applicants must be able to demonstrate that all subcontractors were selected according to an applicant's institutional requirements for the selection of subcontractors (competitive selection or sole source justification).

CBDA retains the right to request that a subcontractor provide cost estimates in writing prior to distribution of grant funds.

CBDA retains the right to request consultant, subcontractor, and/or outside service provider cost estimates in writing prior to distribution of grant funds.

### *Indirect Costs (Overhead)*

Indirect costs are overhead expenses incurred by the applicant organization as a result of the project but are not easily identifiable with a specific project. The indirect cost rate consists of a reasonable percentage of all costs to run the agency or organization while completing the project. List the cost and items associated with indirect costs. (These items may include general office expenses such as rent, office equipment, administrative staff, operational costs, etc. Generally these items are represented by the applicant through a predetermined percentage or surcharge separate from other specific costs of items necessary to complete a specific task or subtask.)

If indirect cost rates are different for State and Federal funds, please identify each rate and the specific items included in the calculation for that rate.

Task 1.1, Quantifying The Occurrence Of (Xeno)Estrogens Using Chemical Analysis:	Justification	Amount
----------------------------------------------------------------------------------	---------------	--------

<b>Labor</b>		
Sedlak, David L., Ph.D.	<i>1.5 months salary at 100% time</i>	<i>14651</i>
Kolodziej, Edward P., Ph.D.	<i>18 months salary at 100% time, 20% of time will be devoted solely to project management duties</i>	<i>75000</i>
*Graduate Student	<i>13.5 months salary at 50% time; 4.5 months salary at 100% time</i>	<i>32990</i>
<b>Task 1.1, Quantifying The Occurrence Of (Xeno)Estrogens Using Chemical Analysis: Benefits</b>	<b>Justification</b>	<b>Amount</b>
Sedlak, David L., Ph.D.	<i>12.7 % benefits rate; full benefits provided</i>	<i>1861</i>
Kolodziej, Edward P., Ph.D.	<i>22% benefits rate; full benefits provided</i>	<i>12750</i>
*Graduate Student	<i>1.3% benefits rate for 50% time, medical and dental benefits only; 3% benefits rate for 100% time, includes retirement contribution; 3 semesters tuition fee remission included</i>	<i>12212</i>
<b>Task 1.1, Quantifying The Occurrence Of (Xeno)Estrogens Using Chemical Analysis: Travel Expenses</b>	<b>Justification</b>	<b>Amount</b>
<i>Other</i>	<i>Project management trip costs, 9 trips for project manager to travel to UC Riverside at \$700 per trip (airfare, lodging, meals)</i>	<i>6300</i>
<i>Conferences</i>	<i>2 Advisory board meetings, \$5100 each, includes stipend (\$1500 per advisory board member), day trip to UC Riverside (2 people at \$250 each), and \$100 for AMS car travel.</i>	<i>10200</i>
<i>Conferences</i>	<i>3 Major conference trips (\$1200 per person per trip estimate for airfare, lodging, and per diem)</i>	<i>3600</i>
<i>Other</i>	<i>mileage and per diem costs for sample site reconnaissance (2 trips) and sampling trips (6 trips for project manager only), estimated at \$300 per trip</i>	<i>2400</i>
<b>Task 1.1, Quantifying The Occurrence Of (Xeno)Estrogens Using Chemical Analysis: Supplies And Expendables</b>	<b>Justification</b>	<b>Amount</b>
<i>Other</i>	<i>GC/MS/MS maintenance costs</i>	<i>7500</i>
<i>Postage/Delivery</i>	<i>Shipping costs</i>	<i>3000</i>
<i>Other</i>	<i>Chemicals</i>	<i>5000</i>
<i>Other</i>	<i>Filtration apparatus, filters, and extraction discs</i>	<i>8000</i>
<i>Other</i>	<i>Glassware</i>	<i>4000</i>
<i>Other</i>	<i>General lab supplies</i>	<i>4000</i>
<i>Other</i>	<i>Vacuum pump</i>	<i>3000</i>
<i>Other</i>	<i>Hazardous waste disposal</i>	<i>3000</i>
<b>Task 1.1, Quantifying The Occurrence Of (Xeno)Estrogens Using Chemical Analysis: Subcontractors</b>	<b>Justification</b>	<b>Amount</b>
*Subcontractor For Sampling Services	<i>Cost for sample collection, including sample site selection (\$1592), sample and field plan preparation (\$3943), 12 monthly sampling events (\$14079 each), and 3 storm event sampling events (\$8744 each)</i>	<i>200715</i>
<b>Task 1.1, Quantifying The Occurrence Of (Xeno)Estrogens Using Chemical Analysis: Equipment</b>	<b>Justification</b>	<b>Amount</b>
GC/MS/MS	<i>Cost of new Thermoquest GC/MS/MS analytical instrument, as current GC/MS/MS analytical instrument will reach end of product lifetime during project and proper GC/MS/MS operation and sensitivity is essential to quantification of (xeno)estrogens. The number of analysis in this project will necessitate a new instrument.</i>	<i>125000</i>

Task 1.1, Quantifying The Occurrence Of (Xeno)Estrogens Using Chemical Analysis: Other Direct	Justification	Amount
Task 1.1, Quantifying The Occurrence Of (Xeno)Estrogens Using Chemical Analysis: Indirect (Overhead)	Justification	Amount
	<i>25% Indirect Cost rate less \$25,000 (non-overhead subcontractor portion)</i>	<i>55726</i>
	<b>Task 1.1 Total</b>	<b>\$590,905</b>
Task 1.2, Quantifying Estrogen Agonists In Water Samples With An In Vitro Bioassay: Labor	Justification	Amount
Schlenk, Daniel, Ph.D.	<i>1.5 months salary at 50% time</i>	<i>6846</i>
*Post-Doctoral Researcher	<i>18 months salary at 50% time</i>	<i>24762</i>
Task 1.2, Quantifying Estrogen Agonists In Water Samples With An In Vitro Bioassay: Benefits	Justification	Amount
Schlenk, Daniel, Ph.D.	<i>12.70% benefit rate x 6846; full benefits</i>	<i>869</i>
*Post-Doctoral Researcher	<i>25% benefit rate x 24762; full benefits</i>	<i>6195</i>
Task 1.2, Quantifying Estrogen Agonists In Water Samples With An In Vitro Bioassay: Travel Expenses	Justification	Amount
Conferences	<i>2 Major conference trips (\$1200 per person per trip estimate for airfare, lodging, and per diem)</i>	<i>2400</i>
Task 1.2, Quantifying Estrogen Agonists In Water Samples With An In Vitro Bioassay: Supplies And Expendables	Justification	Amount
Other	<i>Reagents and disposable lab supplies</i>	<i>1500</i>
Other	<i>Glassware and aquaria</i>	<i>1500</i>
Other	<i>Enzyme-linked immunoassay kits</i>	<i>3750</i>
Other	<i>Service contracts for instrumentation</i>	<i>750</i>
Telephone	<i>Communications</i>	<i>750</i>
Task 1.2, Quantifying Estrogen Agonists In Water Samples With An In Vitro Bioassay: Subcontractors	Justification	Amount
	<i>No subcontractor was assigned to this task.</i>	
Task 1.2, Quantifying Estrogen Agonists In Water Samples With An In Vitro Bioassay: Equipment	Justification	Amount
Task 1.2, Quantifying Estrogen Agonists In Water Samples With An In Vitro Bioassay: Other Direct	Justification	Amount
Task 1.2, Quantifying Estrogen Agonists In Water Samples With An In Vitro Bioassay: Indirect (Overhead)	Justification	Amount
	<i>UC Riverside 25% IDC rate</i>	<i>12329</i>
	<b>Task 1.2 Total</b>	<b>\$61,651</b>
Task 1.3, Development Of In Vivo Bioassays For Feminization Of Early Life-Stage Salmon: Labor	Justification	Amount

Schlenk, Daniel, Ph.D.	<i>1.5 months salary at 50% time</i>	6846
*Post-Doctoral Researcher	<i>18 months salary at 50% time</i>	24762
Task 1.3, Development Of In Vivo Bioassays For Feminization Of Early Life-Stage Salmon: Benefits	Justification	Amount
Schlenk, Daniel, Ph.D.	<i>12.70% benefit rate x 6846; full benefits</i>	869
*Post-Doctoral Researcher	<i>25% benefit rate x 24762; full benefits</i>	6195
Task 1.3, Development Of In Vivo Bioassays For Feminization Of Early Life-Stage Salmon: Travel Expenses	Justification	Amount
Mileage	<i>mileage and travel costs associated with salmon culturing, including egg procurement and transport costs (4 trips at \$300 per trip)</i>	1200
Task 1.3, Development Of In Vivo Bioassays For Feminization Of Early Life-Stage Salmon: Supplies And Expendables	Justification	Amount
Other	<i>Reagents and disposable lab supplies</i>	1500
Other	<i>Glassware and aquaria</i>	1500
Other	<i>Enzyme-linked immunoassay kits</i>	3750
Other	<i>Service contracts for instrumentation</i>	750
Task 1.3, Development Of In Vivo Bioassays For Feminization Of Early Life-Stage Salmon: Subcontractors	Justification	Amount
<i>No subcontractor was assigned to this task.</i>		
Task 1.3, Development Of In Vivo Bioassays For Feminization Of Early Life-Stage Salmon: Equipment	Justification	Amount
Task 1.3, Development Of In Vivo Bioassays For Feminization Of Early Life-Stage Salmon: Other Direct	Justification	Amount
Task 1.3, Development Of In Vivo Bioassays For Feminization Of Early Life-Stage Salmon: Indirect (Overhead)	Justification	Amount
	<i>25% IDC rate</i>	11842
<b>Task 1.3 Total</b>		\$59,214
Task 2.1, Targeted Sampling At Selected Locations: Labor	Justification	Amount
Sedlak, David L., Ph.D.	<i>1.5 months salary at 80% time</i>	12527
Kolodziej, Edward P., PhD.	<i>18 months salary at 80% time, 20% of time will be devoted solely to project management duties</i>	60000
*Graduate Student	<i>13.5 months salary at 40% time; 4.5 months salary at 80% time</i>	27099
Task 2.1, Targeted Sampling At Selected Locations: Benefits	Justification	Amount
Sedlak, David L., Ph.D.	<i>12.7 % benefits rate; full benefits provided</i>	1591
Kolodziej, Edward P., PhD.	<i>22% benefits rate; full benefits provided</i>	10200
*Graduate Student	<i>1.3% benefits rate for 50% time, medical and dental benefits only; 3% benefits rate for 100% time, includes retirement contribution; 2.4 semesters tuition fee remission included</i>	11036
Task 2.1, Targeted Sampling At Selected Locations: Travel Expenses	Justification	Amount
		6300

	<i>Project management trip costs, 9 trips for project manager to travel to UC Riverside at \$700 per trip (airfare, lodging, meals)</i>	
<i>Other</i>		
<i>Conferences</i>	<i>1 Advisory board meeting, \$5100 each, includes stipend (\$1500 per advisory board member), day trip to UC Riverside (2 people at \$250 each), and \$100 for AMS car travel.</i>	<i>5100</i>
<i>Other</i>	<i>mileage and per diem costs for monthly sample site sampling trips (4 trips for project manager only), estimated at \$300 per trip</i>	<i>1200</i>
<i>Other</i>	<i>mileage and per diem costs for intensive watershed sampling , including 2 reconnaissance trips (\$300 each), and 4 sample collection events (4 people per sampling event, \$500 per trip total)</i>	<i>2600</i>
<i>Conferences</i>	<i>2 Major conference trips (\$1200 per person per trip estimate for airfare, lodging, and per diem)</i>	<i>2400</i>
<hr/>		
<b>Task 2.1, Targeted Sampling At Selected Locations: Supplies And Expendables</b>	<b>Justification</b>	<b>Amount</b>
<i>Reproduction</i>	<i>GC/MS/MS maintence</i>	<i>7500</i>
<i>Other</i>	<i>Chemicals</i>	<i>5000</i>
<i>Other</i>	<i>Filtration apparatus, filters, and extraction discs</i>	<i>8000</i>
<i>Postage/Delivery</i>	<i>Shipping costs</i>	<i>3000</i>
<i>Other</i>	<i>Glassware</i>	<i>2000</i>
<i>Other</i>	<i>Sampling containers</i>	<i>2000</i>
<i>Other</i>	<i>General lab supplies</i>	<i>4000</i>
<i>Office/Presentation Supplies</i>	<i>Office supplies and report preparation</i>	<i>3000</i>
<i>Other</i>	<i>Hazardous waste disposal</i>	<i>3000</i>
<hr/>		
<b>Task 2.1, Targeted Sampling At Selected Locations: Subcontractors</b>	<b>Justification</b>	<b>Amount</b>
<i>*Subcontractor For Sampling Services</i>	<i>Cost for sample collection, including 3 monthly sampling events (\$14079 each), and 1 storm event sampling events (\$8744 each)</i>	<i>50981</i>
<hr/>		
<b>Task 2.1, Targeted Sampling At Selected Locations: Equipment</b>	<b>Justification</b>	<b>Amount</b>
<hr/>		
<b>Task 2.1, Targeted Sampling At Selected Locations: Other Direct</b>	<b>Justification</b>	<b>Amount</b>
<hr/>		
<b>Task 2.1, Targeted Sampling At Selected Locations: Indirect (Overhead)</b>	<b>Justification</b>	<b>Amount</b>
	<i>25% IDC rate on all items except tuition, subcontract</i>	<i>41763</i>
		<b>Task 2.1 Total</b>
		<b>\$270,297</b>
<hr/>		
<b>Task 2.2, Toxicity Identification Evaluation: Labor</b>	<b>Justification</b>	<b>Amount</b>
<i>Sedlak, David L., Ph.D.</i>	<i>1.5 months salary at 20% time</i>	<i>3132</i>
<i>Schlenk, Daniel, Ph.D.</i>	<i>1.5 months salary at 20% time</i>	<i>2739</i>
<i>Kolodziej, Edward P., Ph.D.</i>	<i>18 months salary at 20% time, 20% of time will be devoted solely to project management duties</i>	<i>15000</i>
<i>*Post-Doctoral Researcher</i>	<i>18 months salary at 20% time</i>	<i>9905</i>
<i>*Graduate Student</i>	<i>13.5 months salary at 10% time; 4.5 months salary at 20% time</i>	<i>6775</i>
<hr/>		
<b>Task 2.2, Toxicity Identification Evaluation: Benefits</b>	<b>Justification</b>	<b>Amount</b>
<i>Sedlak, David L., Ph.D.</i>	<i>12.7 % benefits rate; full benefits provided</i>	<i>398</i>

Schlenk, Daniel, Ph.D.	<i>12.7% benefits rate; full benefits</i>	348
Kolodziej, Edward P., PhD.	<i>22% benefits rate; full benefits provided</i>	2550
*Post-Doctoral Researcher	<i>25% benefits rate; full benefits</i>	2476
*Graduate Student	<i>1.3% benefits rate for 50% time, medical and dental benefits only; 3% benefits rate for 100% time, includes retirement contribution; 0.6 semesters tuition fee remission included</i>	2759
<b>Task 2.2, Toxicity Identification Evaluation: Travel Expenses</b>	<b>Justification</b>	<b>Amount</b>
<b>Task 2.2, Toxicity Identification Evaluation: Supplies And Expendables</b>	<b>Justification</b>	<b>Amount</b>
<i>Other</i>	<i>Reagents and disposable lab supplies</i>	1500
<i>Other</i>	<i>Glassware and aquaria</i>	1500
<i>Other</i>	<i>Enzyme-linked immunoassay kits</i>	3750
<i>Other</i>	<i>Service contracts for instrumentation</i>	750
<b>Task 2.2, Toxicity Identification Evaluation: Subcontractors</b>	<b>Justification</b>	<b>Amount</b>
Snyder, Shane A., Ph.D.	<i>Dr. Snyder has specialized analytical capabilities</i>	20000
<b>Task 2.2, Toxicity Identification Evaluation: Equipment</b>	<b>Justification</b>	<b>Amount</b>
<b>Task 2.2, Toxicity Identification Evaluation: Other Direct</b>	<b>Justification</b>	<b>Amount</b>
<b>Task 2.2, Toxicity Identification Evaluation: Indirect (Overhead)</b>	<b>Justification</b>	<b>Amount</b>
	<i>25% IDC rate on all items except tuition</i>	17739
	<b>Task 2.2 Total</b>	\$91,321
<b>Task 2.3, Conduct In Vivo Bioassays On Selected Samples: Labor</b>	<b>Justification</b>	<b>Amount</b>
Schlenk, Daniel, Ph.D.	<i>1.5 months salary at 80% time</i>	10954
*Post-Doctoral Researcher	<i>18 months salary at 80% time</i>	39619
<b>Task 2.3, Conduct In Vivo Bioassays On Selected Samples: Benefits</b>	<b>Justification</b>	<b>Amount</b>
Schlenk, Daniel, Ph.D.	<i>12.7% benefit rate; full benefits</i>	1391
*Post-Doctoral Researcher	<i>25% benefit rate; full benefits</i>	9905
<b>Task 2.3, Conduct In Vivo Bioassays On Selected Samples: Travel Expenses</b>	<b>Justification</b>	<b>Amount</b>
<i>Conferences</i>	<i>2 Major conference trips (\$1200 per person per trip estimate for airfare, lodging, and per diem)</i>	2400
<b>Task 2.3, Conduct In Vivo Bioassays On Selected Samples: Supplies And Expendables</b>	<b>Justification</b>	<b>Amount</b>
<i>Other</i>	<i>Reagents and disposable lab supplies</i>	1500
<i>Other</i>	<i>Glassware and aquaria</i>	1500
<i>Other</i>	<i>Enzyme-linked immunoassay kits</i>	3750
<i>Other</i>	<i>Service contracts for instrumentation</i>	750
	<b>Justification</b>	<b>Amount</b>

Task 2.3, Conduct In Vivo Bioassays On Selected Samples: Subcontractors		
<i>No subcontractor was assigned to this task.</i>		
Task 2.3, Conduct In Vivo Bioassays On Selected Samples: Equipment	<b>Justification</b>	<b>Amount</b>
Task 2.3, Conduct In Vivo Bioassays On Selected Samples: Other Direct	<b>Justification</b>	<b>Amount</b>
Task 2.3, Conduct In Vivo Bioassays On Selected Samples: Indirect (Overhead)	<b>Justification</b>	<b>Amount</b>
	<i>25% IDC rate</i>	<i>17492</i>
	<b>Task 2.3 Total</b>	<b>\$89,261</b>
Task 2.4, Analyze Extent Of Feminization With Other Fish Species: Labor	<b>Justification</b>	<b>Amount</b>
Spies, Robert B., PhD.		<i>134500</i>
Task 2.4, Analyze Extent Of Feminization With Other Fish Species: Benefits	<b>Justification</b>	<b>Amount</b>
Spies, Robert B., PhD.		
Task 2.4, Analyze Extent Of Feminization With Other Fish Species: Travel Expenses	<b>Justification</b>	<b>Amount</b>
Task 2.4, Analyze Extent Of Feminization With Other Fish Species: Supplies And Expendables	<b>Justification</b>	<b>Amount</b>
Task 2.4, Analyze Extent Of Feminization With Other Fish Species: Subcontractors	<b>Justification</b>	<b>Amount</b>
*Subcontractor For Sampling Services		
Task 2.4, Analyze Extent Of Feminization With Other Fish Species: Equipment	<b>Justification</b>	<b>Amount</b>
Task 2.4, Analyze Extent Of Feminization With Other Fish Species: Other Direct	<b>Justification</b>	<b>Amount</b>
Task 2.4, Analyze Extent Of Feminization With Other Fish Species: Indirect (Overhead)	<b>Justification</b>	<b>Amount</b>
	<b>Task 2.4 Total</b>	<b>\$134,500</b>
	<b>Grand Total</b>	<b>\$1,297,149</b>

X The indirect costs may change by more than 10% if federal funds are awarded for this proposal.

What is the total of non-federal funds requested? **100%**

# Identifying the Causes of Feminization of Chinook Salmon in the Sacramento and San Joaquin River System

## 1. Project Purpose

### 1.1 Background

Starting in the early 1990s, scientists began to report the presence of feminized male fish in rivers around the world. Sex reversal in male fish, which is a form of endocrine disruption, has attracted the attention of scientists, policymakers and the general public. Most research on feminization of fish has focused on rivers in which the discharge of municipal wastewater accounts for a significant fraction of the overall flow. In these systems, a large percentage of the male fish often exhibit elevated plasma levels of the lipoprotein vitellogenin and express egg cells in their testes (Purdom *et al.* 1994, Harries *et al.* 1996, Jobling *et al.* 1998). Related studies have demonstrated that the feminization of male fish in these systems usually is attributable to the presence in wastewater effluent of trace concentrations of steroid hormones, such as ethinyl estradiol, 17 $\beta$ -estradiol and estrone (Desbrow *et al.* 1998, Snyder *et al.* 1999).

Although the presence of steroid hormones in wastewater effluent usually explains the feminization of male fish in urban surface waters, it does not explain the feminization of Chinook salmon (*Oncorhynchus tshawytscha*) observed by Williamson and May (2002). These researchers analyzed over 400 fin clips from adult Chinook salmon collected at 13 locations in the Sacramento and San Joaquin River watersheds. In these samples, the frequency of genotypically male salmon exhibiting complete sex reversal, as indicated by the presence of ovaries in salmon expressing a Y-chromosome specific marker, was as high as 38% (Table 1). Nagler *et al.* (2001) reported similar findings in the Hanford Reach of the Columbia River (Table 1) where up to 84% of the genotypically male fish exhibited female characteristics. Hatchery-raised salmon from the Sacramento, San Joaquin and Columbia Rivers also exhibited feminization, but at a lower frequency, while none of the salmon collected from a hatchery in British Columbia showed sex reversal.

Feminization of genotypically male fish can be caused by temperature fluctuations during development and by exposure to chemicals that bind with estrogen receptors (Nakamura *et al.* 1998, Devlin and Nakahama 2002). In the case of Chinook salmon, fluctuations in water temperature do not alter phenotypic sex ratios (Nagler *et al.* 2003, Chowen *et al.*, 2004) and no other environmental variables are known to cause feminization. Therefore, scientists studying this phenomenon believe that the most likely cause is exposure to either endogenous estrogens or chemical contaminants that affect the estrogen hormone axis (Nagler *et al.* 2003, Williamson and May 2002). These two classes of chemicals are known as (xeno)estrogens.

The Sacramento and San Joaquin watersheds likely contain a variety of chemical contaminants known to act as (xeno)estrogens. For example, dietary exposures or injections of steroid hormones and pesticides can cause feminization of salmon (Devlin and Nakahama 2002). Although waterborne exposures to sewage effluent also can cause feminization of Chinook salmon (Afonso *et al.* 2002), few *in vivo* studies involving waterborne exposures of Chinook salmon to specific contaminants have been performed. However, waterborne exposure to (xeno)estrogens can cause feminization of the closely related species, rainbow trout (*Oncorhynchus mykiss*). Observations with rainbow trout provide insight into the types of

**Table 1.** Frequency of male wild and hatchery Chinook salmon exhibiting sex reversal.

Location	Watershed	Sex-Reversal	Reference
Mokelumne River	San Joaquin River	38%	1
Battle Creek	Sacramento River	35%	1
Yuba River	Sacramento River	25%	1
Merced River	San Joaquin River	24%	1
American River	Sacramento River	20%	1
Feather River	Sacramento River	20%	1
Stanislaus River	San Joaquin River	12%	1
Tuolumne River	San Joaquin River	12%	1
Clear Creek	Sacramento River	6%	1
Hanford Reach	Columbia River	53%	2
Yakima River	Columbia River	33%	2
Mokelumne River Hatchery	San Joaquin River	4%	1
Merced River Hatchery	San Joaquin River	14%	1
Feather River Hatchery	Sacramento River	0%	1
Nimbus Hatchery	Sacramento River	12%	1
Priest Rapids Hatchery	Columbia River	62%	2
Big Qualicum Hatchery	Big Qualicum River (BC)	0%	3

1 = Williamson and May (2002); 2= Chowen and Nagler (2004); 3= Alfonso *et al.* (2002)

chemical contaminants that might be responsible for feminization of Chinook salmon. For example, induction of vitellogenin production has been observed upon exposure of juvenile rainbow trout to steroid hormone concentrations as low as 1 ng/L (Table 2). Waterborne exposure to higher concentrations of nonylphenol and octylphenol, common metabolites of non-ionic detergents, also can induce vitellogenin production in rainbow trout. Although no data are available on effects on fish, certain pyrethroid pesticides and their metabolites (*e.g.*, 3-phenoxybenzyl alcohol) bind to estrogen receptors used in the yeast estrogen screen (YES) *in vitro* bioassay. Although less information is available on the waterborne exposure route, metabolites of the persistent organic pollutants DDT and PCBs (DDE and hydroxylated PCBs, such as 4-hydroxy-2'4'6'-trichlorobiphenyl) also can induce vitellogenin production when exposure occurs via food or by direct injection into the fish.

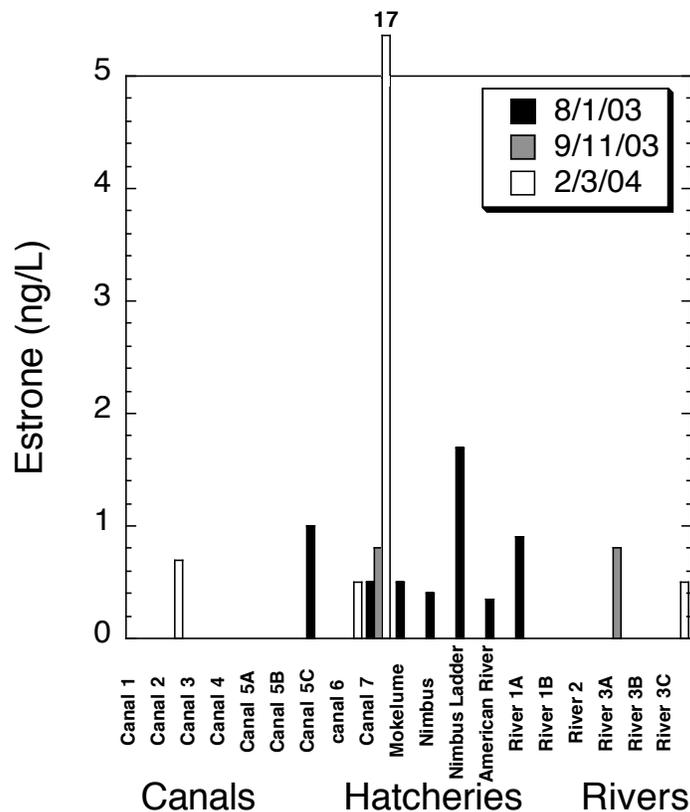
Salmon that spawn in the Sacramento River and San Joaquin River watersheds are exposed to many of the (xeno)estrogens listed in Table 2. Steroid hormones are present in municipal wastewater effluent (Huang and Sedlak 2001) and in agricultural wastes (Hanselman *et al.* 2003). For example, we recently reported the presence of the steroid hormone estrone at concentrations up to 17 ng/L in drainage canals in an area of California's Central Valley with a high density of dairy farms (Figure 1). Concentrations of steroid hormones were higher during or shortly after winter storms suggesting that runoff from agricultural operations was an important source of steroid hormones to surface waters. Steroid hormones also were detected in water discharged by fish hatcheries along the American and Mokelumne Rivers (Kolodziej *et al.* 2004) indicating that aquaculture may also play a role in feminization of salmon.

**Table 2.** Chemical contaminants capable of causing feminization of male fish or binding to estrogen receptors.

Contaminant	Threshold Concentration	Assay	Reference
Ethinyl estradiol	1 ng/L	Rainbow trout	1
17 $\beta$ -estradiol	10 ng/L	Rainbow trout	1
Estrone	44 ng/L	Rainbow trout	1
4-nonylphenol	10 $\mu$ g/L	Rainbow trout	2
4-octylphenol	3 $\mu$ g/L	Rainbow trout	2
3-phenoxybenzyl alcohol	10 $\mu$ g/L	YES Assay	3
4,4'-DDE	NA	Rainbow trout	4
4-hydroxy-2'4'6'-trichlorobiphenyl	NA	Rainbow trout	5

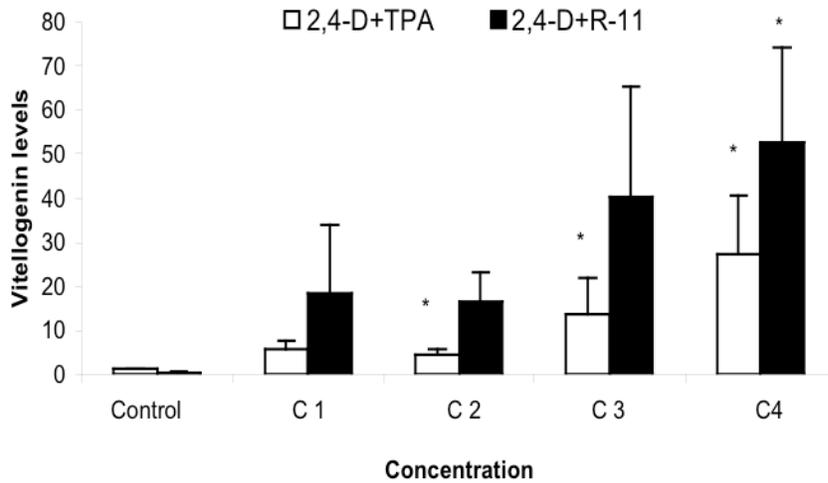
NA = Not applicable

1 = Routledge *et al.* (1998); 2 = Jobling *et al.* (1996); 3 = Tyler *et al.* (2000) ; 4 = Donohoe and Curtis (1996); 5 = Carlson and Williams (2001)



**Figure 1:** Concentrations of the steroid hormone estrone in dairy farm drainage canals, fish hatchery effluent and the Merced River (data from Kolodziej *et al.* 2004).

Runoff from farms that use pesticides also may contain xenoestrogens. Although most pesticides do not cause feminization of fish at the concentrations expected in agricultural runoff, elevated concentrations of estrogen-binding compounds have been detected in agricultural areas and it has been hypothesized that the detergents used to deliver the pesticides are the causative agents (Hamers *et al.* 2003). Consistent with this hypothesis, preliminary data from the Schlenk laboratory indicated feminization of Rainbow trout exposed to two commercial detergents (TPA and R-11) and two aquatic herbicides (2,4 D and trichlopyr) that are commonly used in concert in the Central Valley in California (see Figure 2). In these experiments, both 2,4-D and R-11 caused vitellogenin induction in exposures when the concentrations of the surfactant and the pesticide were comparable. The ability of the surfactant R-11 to induce vitellogenin production is most likely attributable to the presence of alkylphenol polyethoxylates, which account for approximately 90% of the mass of the R-11 formulation. In surface waters of the Central Valley and Delta, the concentration of alkylphenol polyethoxylates is likely to be significantly higher than that of the xenoestrogenic pesticides. Another interesting feature of the data in Figure 2 is that the feminization response was greater than additive when the fish were treated with surfactant and pesticide together at environmentally relevant doses, but less than additive when the fish were exposed to higher concentrations.



**Figure 2:** Vitellogenin levels in juvenile rainbow trout exposed to a mixture of 2,4-D and R-11 or TPA. \* indicates significant difference in vitellogenin levels from control fish. Exposure conditions as indicated below:

Dose	Concentrations of chemicals	
	TPA (mg/L)+2,4-D (mg/L)	R-11 (mg/L)+2,4-D (mg/L)
C 1	0.00048 TPA + 0.00164 2,4-D	0.00089 R-11 + 0.00164 2,4-D
C 2	0.0048 TPA + 0.0164 2,4-D	0.0089 R-11 + 0.0164 2,4-D
C 3	0.048 TPA + 0.164 2,4-D	0.089 R-11 + 0.164 2,4-D
C 4	0.48 TPA + 1.64 2,4-D	0.89 R-11 + 1.64 2,4-D

Surfactants are not the only xenoestrogens associated with pesticide use that could contribute to feminization of salmon; the pyrethroid pesticides and their metabolites are heavily utilized in Central Valley agriculture and they have demonstrated estrogenic activity. Over 130,000 kg of permethrin was applied in California in 2002 (Weston *et al.* 2004) and it is possible that these compounds also contribute to feminization of salmonids.

Salmon that spawn in the Sacramento and San Joaquin River watersheds also may be exposed to metabolites of persistent organic pollutants (POPs) listed in Table 2 (*i.e.*, DDE and hydroxylated PCBs) through their diets or through contact with contaminated sediments. As female adult salmon return to their home watersheds to spawn, they concentrate these hydrophobic organic compounds in their fat (Debruyne *et al.* 2004) and relatively high concentrations of these compounds may be transferred to their eggs. Thus, exposure of developing fish to elevated concentrations of xenoestrogens could occur through maternal transfer from fish that are exposed to contaminated food in the ocean or the Bay/Delta.

In addition to causing feminization, many of the chemical contaminants listed in Table 2 also could impact salmon reproductive success and survival through other mechanisms. For example, exogenous steroid hormones can interfere with chemical communication in salmon, which is important to the timing of reproduction (Kolodziej *et al.* 2003). Also, Arsenault *et al.* (2004) have shown that growth and insulin-like growth factor-I levels are depressed in juvenile Atlantic salmon (*Salmo salar*) exposed to environmentally relevant concentrations of 4-nonylphenol or 17 $\beta$ -estradiol. Finally, hydrophobic xenoestrogens, such as DDE, can cause immunosuppression in salmon (Miltson *et al.* 2003). Although the main focus of this research project is feminization of salmonids, information on the source and behavior of these contaminants also will be useful in the evaluation of other, potentially harmful chemical stressors of potential importance to salmon in the Sacramento River, San Joaquin River and Delta ecosystems.

The observation of feminized fish in the Sacramento and San Joaquin Rivers raises several critical questions that are particularly relevant to the CALFED Program:

- Has feminization of male salmon played a role in salmon population declines?
- Will feminization affect efforts to protect and restore Chinook salmon and other key fish species such as the Delta smelt (*Hypomesus transpacificus*), Sacramento splittail and tule perch (*Hysterocarpus traski*)?
- Are chemical contaminants in water responsible for feminization of fish in the Bay/Delta ecosystem, and if so, which chemicals are responsible?
- Do (xeno)estrogens cause other related, subtle effects on sensitive fish species?
- Are there any cost-effective actions that can be used to reduce exposure of fish to (xeno)estrogens?

## 1.2 Project Goals

The main purpose of this research project is to identify the agents responsible for feminization of salmon in waters that discharge to the San Francisco-San Joaquin Delta. Through a combination of field sampling, state-of-the-art chemical analyses and laboratory bioassays, we will identify and quantify chemical contaminants capable of causing feminization of salmon in waters that flow through the Delta. By combining chemical analyses and bioassays, we will identify sources of contaminants and identify gaps in our knowledge about the factors that result in feminization of fish. Results obtained for salmon also will provide insight into the potential importance of feminization to other key fish species native to the Delta and its tributaries.

After the contaminants that could be responsible for feminization are identified, we will be in an excellent position to assess the potential effects of these contaminants on CALFED's efforts to protect and restore salmon. Our study will provide data that will assist CALFED and CALFED-affiliated scientists and stakeholders in future efforts to restore salmon and to compare the role of upstream stresses from chemical contaminants to other stressors of salmonids in the Delta region.

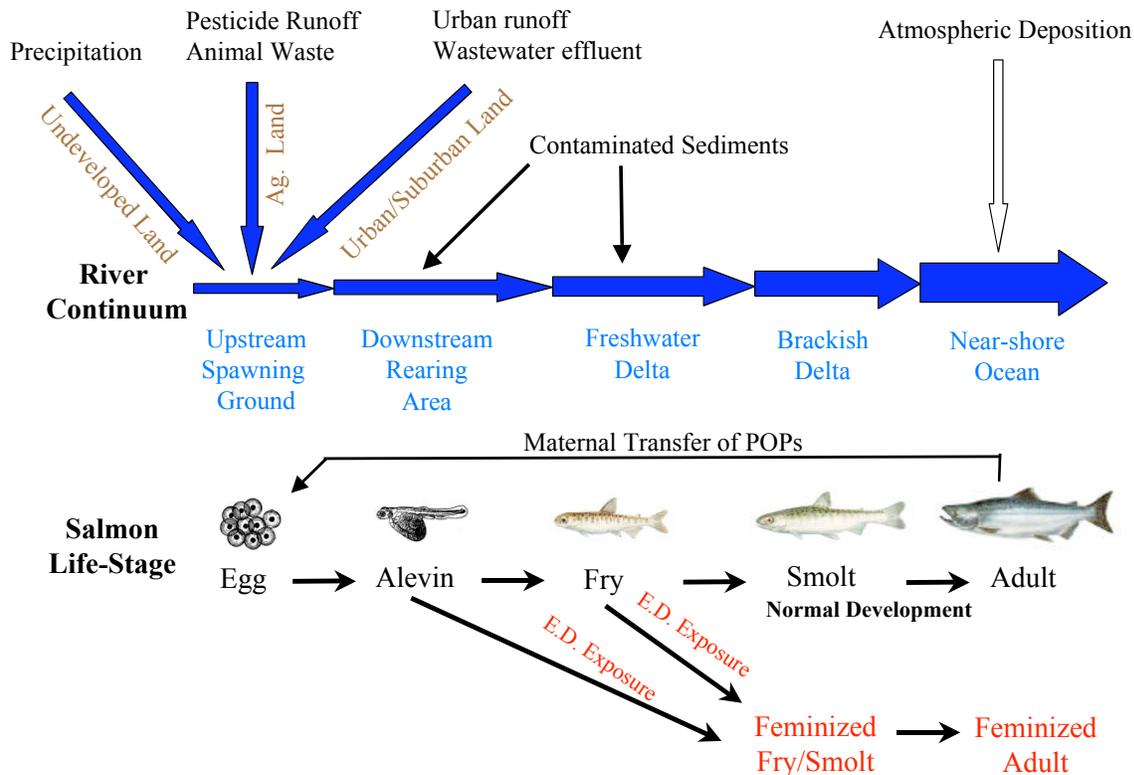
A final objective of the proposed research is to identify cost-effective strategies for minimizing the exposure of fish to (xeno)estrogens. After completion of this research, it may be possible to identify simple management tools to alleviate some of the stress that is attributable to these contaminants. For example, if the runoff from animal waste handling operations causes wet-weather pulses of steroid hormones in surface waters, it might be appropriate to direct effort into better control of surface runoff. Alternatively, if fish feminization is related to urban sources, resources could be dedicated to better control of sources such as urban stormwater runoff.

## 1.3 Conceptual Model

The conceptual model (Figure 3) targets the early life stages (*i.e.*, egg, alevin, fry) of Chinook salmon as critical life stages when exposure to chemical contaminants can result in permanent sex reversal. Previous research (Goetz *et al.* 1979, Hunter *et al.* 1986) has shown that this life stage is critical to feminization of male salmon because it is the period prior to sexual differentiation and it is during this lifestage that chemical contaminants can cause sex reversal. After a male alevin or fry is feminized, it will continue to develop and exhibit characteristics of a sex-reversed or feminized male during its entire lifecycle. Therefore, we will focus our research efforts on these critical early life stages to identify sources of (xeno)estrogens.

The conceptual model will guide the selection of sampling locations and times. Upon hatching, the salmon alevin in the Bay/Delta system live among the gravel and rocks of the spawning areas prior to their downstream migration (as fry) to rearing areas in the lower tributaries and the freshwater Delta (Allan and Hassler 1986). For fall-run Chinook salmon, the most abundant salmonid in the Bay-Delta ecosystem and the species in which sex-reversal has been documented (Williamson *et al.* 2001), the critical period in which early life-stage fish can be exposed is December through May. For spring-run Chinook salmon, the critical life stage occurs between October and March. Therefore, we will focus our efforts on the region of the Sacramento and San Joaquin River watersheds between the freshwater delta and the upstream salmon spawning areas during the critical periods when exposure may occur. To assess the effects of chemical contaminants on other salmonids, samples also will be collected throughout the year at a lower frequency.

The conceptual model considers the concentrations of contaminants and the relative flows of water from agricultural, urban/suburban and undeveloped watersheds. Within each of the different sources of water, a variety of different factors (*e.g.*, agricultural practices, types of chemicals used, volume of precipitation) will determine the concentrations of contaminants and relative flows. Therefore, sampling locations will be selected to represent specific sources of contaminants as described in Section 2.1. In addition to the different sources of waterborne contaminants, the conceptual model also considers exposure of early life-stage salmon to contaminants derived from the maternal transfer of POPs to the eggs and sediment-bound contaminants. The main exposure of the adult female salmon to POPs occurs in the ocean, where long-range atmospheric transport and bioaccumulation result in elevated concentrations of hydrophobic contaminants and their metabolites in salmon prey. Exposure of the early life-stage salmon to contaminated sediments occurs during the period in which the alevin and fry spend most of their time hiding from predators in rocks and gravel. Although sediment exposures are not expected to be a major source of (xeno)estrogens to salmonids, this potential source will be evaluated through laboratory exposures to sediments and analysis of samples from reference watersheds in undeveloped areas.



**Figure 3.** Conceptual model for feminization of Chinook salmon in waters of California's Central Valley and Delta.

## 2. Project Description

The main hypothesis of the proposed research project is that feminization of fish observed in the Delta and its tributaries is due to the presence of chemical contaminants originating in agricultural, urban, and suburban areas in the upstream watershed. To test this hypothesis and to realize the project goals, the research will be conducted by an interdisciplinary team of environmental chemists and ecotoxicologists (see attached CVs for details). The project will be conducted in two phases, which are described below. The descriptions of tasks are followed by details of the experimental methods and project organization.

### 2.1 Phase I: Quantification of (xeno)estrogens

In the first phase of the research project we will quantify the concentrations of (xeno)estrogens in the Delta and its tributaries. We also will develop and test protocols for *in vivo* bioassays of Chinook salmon exposed to waterborne contaminants.

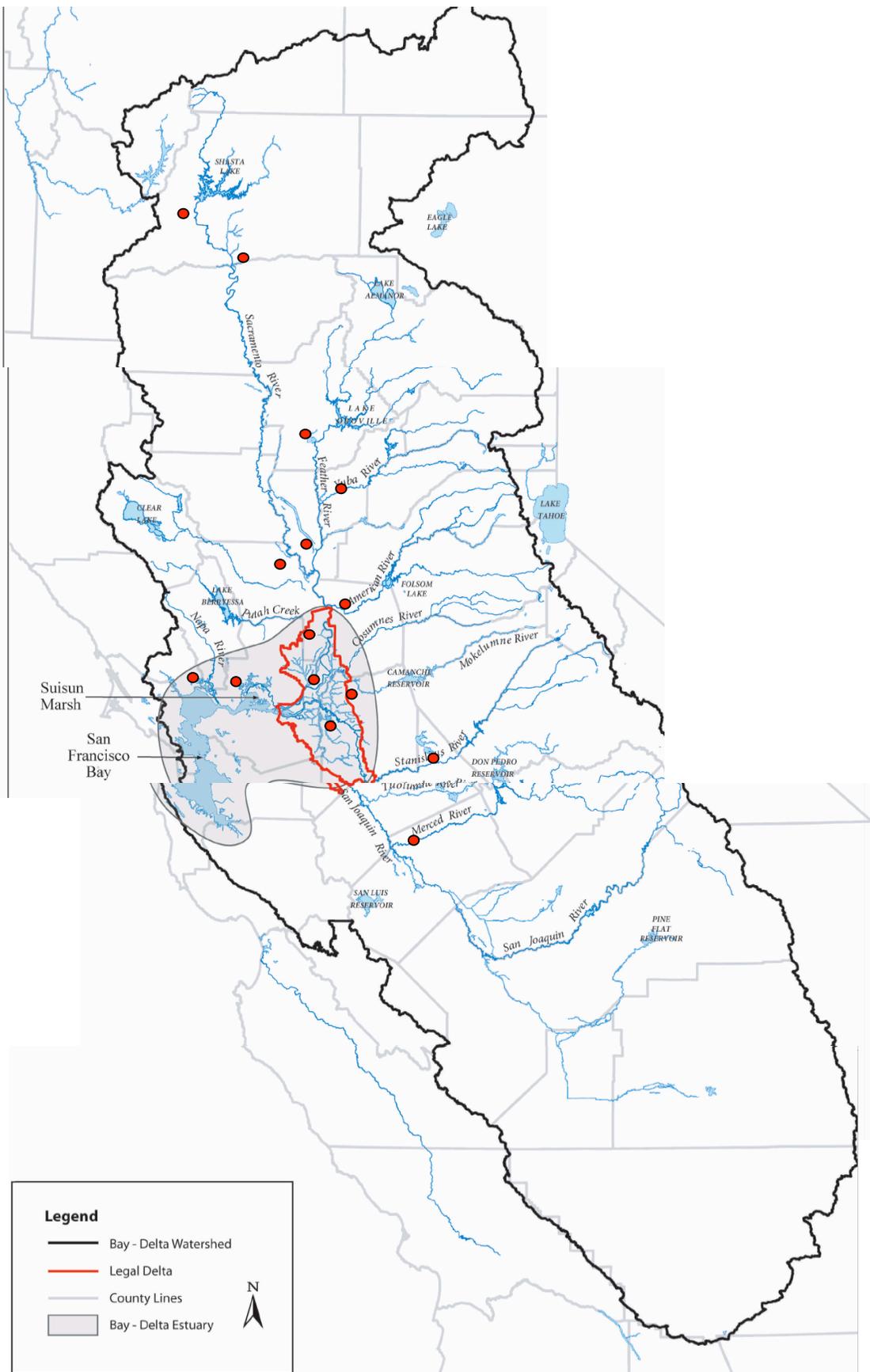
#### *Task 1.1: Quantifying the occurrence of (xeno)estrogens using chemical analysis*

Although a variety of different chemicals that are capable of causing feminization of male fish are used in California, few measurements are available on contaminant concentrations in locations where early life-stage salmon exist. To obtain a better understanding of the occurrence and concentrations of (xeno)estrogens, samples will be collected from locations throughout the Sacramento River, San Joaquin River and freshwater Delta. Likely sampling sites are depicted in Figure 4.

The final selection of sites will depend upon accessibility of sampling sites and detailed evaluations of land use that could affect contaminant sources. Evaluation of land use classifications, which will be accomplished through analysis of census and planning data, will help us to identify surface waters where discharges are predominantly associated with undeveloped land, agricultural and urban/suburban land uses. Because different types of agricultural practices may result in significant variation in the nature and timing of contaminants releases, we will conduct a detailed evaluation of pesticide use in agricultural areas. As part of this analysis, we will use a GIS-based mapping tool that plots annual use patterns of specific types of pesticides at a one mile spatial resolution. The developer of the model, Dr. Susan Kegley, has generously agreed to help us implement the model as part of this project.

During Phase I, the sample collection frequency will be biased towards winter wet weather periods. Monthly base-flow samples will be analyzed from each site during the period from December through April. Samples also will be collected immediately after two storm events to catch runoff-related pulses of contaminants. To assess dry-weather flows, samples will be collected during June, August and October.

Water samples will be analyzed for a suite of (xeno)estrogens hypothesized to be responsible for feminization of salmon in California's Central Valley and Delta, including steroid hormones, alkylphenols and selected pesticides. Analytical methods to be used for quantification of these compounds are described in section 2.3.2.



**Figure 4:** Tentative sampling sites for Phase I.

*Task 1.2: Quantifying estrogen agonists in sample extracts with an in vitro bioassay*

The chemical analyses described in Task 1.1 may not identify all of the chemicals that could be responsible for feminization of salmon. To quantify the total concentration of estrogen agonists (*i.e.*, compounds capable of binding to the estrogen receptor), we will use the *in vitro* YES assay to quantify compounds that are capable of binding to the estrogen receptor in the extracts from water samples being analyzed for chemical contaminants. Comparison of results of the chemical analyses and the YES assay will be used to identify water sources in which unknown (xeno)estrogens are present.

*Task 1.3: Development of in vivo assays for feminization in salmonids*

To assess the potential for water and sediment samples to cause feminization, early life-stage Chinook salmon will be exposed to selected water samples. Feminization will be determined by measurement of vitellogenin in male fish during Phase II of the project. Prior to conducting exposure studies with environmental samples, the salmon bioassay will be developed and validated as part of Task 1.3. In the first stage of these development activities, we will conduct dose/response studies with waterborne exposures to three known (xeno)estrogens (*i.e.*, estrone, 4-nonylphenol and 3-phenoxybenzyl alcohol). These studies will identify threshold values for feminization of early life stage salmon attributable to exposure to these compounds. In addition, we will attempt to adapt the *in vivo* salmon bioassay to assess contaminated sediments by using river sediments from a reference site spiked with different concentrations of DDE. Although these data are being collected mainly to support research in Phase II, they will provide information that will be of great interest to scientists concerned with feminization of salmon because it will provide a means for assessing the relative potency of waterborne exposures of (xeno)estrogens in rainbow trout and Chinook salmon.

## **2.2 Phase II: Identification of Causative Agents**

Data obtained in the Phase I will provide us with an understanding of the temporal and spatial distributions of (xeno)estrogens. During Phase II, we will further characterize spatial and temporal variations in concentrations of (xeno)estrogens and will expand the analytical methods to identify (xeno)estrogens that were not analyzed in Phase I. We will use the *in vivo* Chinook salmon bioassay developed in the Phase I to verify the relationships between chemical analyses and *in vitro* bioassays and their potential to cause feminization of male fish. We also will expand our analyses to include an assessment of the impacts of (xeno)estrogens in other important fish species, including Delta smelt and tule perch. Specific activities associated with each task are described below.

*Task 2.1: Targeted sampling at selected locations*

After completing Phase I, sampling locations will be identified at which elevated concentrations of (xeno)estrogens and/or estrogen-binding activity (as evidenced by the YES assay) are present. To obtain further insight into the identities, sources and temporal distributions of these contaminants, additional sampling will be conducted at and near these sites during the second phase of the project. For example, if elevated concentrations of nonylphenol are detected during wet weather in one of the streams that receives considerable inputs of agricultural runoff, a concentrated sampling program will be employed in that watershed to assess potential upstream contaminant sources. Such an effort would concentrate on the

relationship between agricultural practices and concentrations of the contaminants through the application of pesticide application databases from the California Department of Pesticide Regulation. Likewise, if elevated concentrations of (xeno)estrogens are detected in surface waters downstream of livestock operations, we will evaluate the role of different management activities (*e.g.*, confined animal feeding operations versus grazing) on (xeno)estrogen concentrations.

As part of this task, we anticipate collecting samples from approximately 25 locations in selected watersheds during three or four concentrated field sampling efforts. In conjunction with the targeted sampling, samples will continue to be collected from the locations sampled during Phase I, but at a lower frequency, as determined by analysis of results from the first phase of the study.

#### *Task 2.2: Toxicity/(xeno)estrogen identification evaluation (TIE)*

Results from the *in vitro* bioassay and the chemical analysis conducted during the first phase of the study are likely to identify locations at which the concentrations of the measured compounds are unable to explain the total signal obtained with the *in vivo* bioassay. To identify the (xeno)estrogens responsible for the signal, we will subject extracts from samples in which the discrepancy was observed to chemical fractionation prior to application of the bioassays. In this phase of the research, we will employ the YES assay and an *in vivo* assay with rainbow trout (*Oncorhynchus mykiss*). Trout will be used instead of juvenile Chinook salmon in this part of the research because salmon eggs will not always be available and the Chinook salmon assays will be more labor intensive than the trout assay, which is already running in our laboratories. This assay has been recently used to identify (xeno)estrogens from wastewater of New York City (Sapozhnikova *et al.*, 2005), and sediments near wastewater outfalls of Los Angeles and Orange County (Schlenk *et al.*, manuscript in review). The fractions showing YES or *in vivo* activity will first be separated by selective elution from solid-phase extraction columns. The fractions in which activity is observed then will be subjected to further separation by high performance liquid chromatography (HPLC). Once the active fractions have been identified with the *in vivo* and *in vitro* bioassays, the extracts will be subjected to gas chromatography/tandem mass spectrometry (GC/MS/MS) and HPLC/MS/MS to identify the causative agent(s). Previous studies have indicated multiple compounds with estrogenic activity in wastewater (Sapozhnikova *et al.*, 2005). If multiple compounds are identified from the samples in the proposed study, relative contributions toward biological activity will be assessed by reconstituting fractions or water with measured concentrations of identified compounds. In this way, causation of identified compounds will be verified.

#### *Task 2.3 Conduct salmonid bioassays on selected samples*

To test the hypothesis that feminization of salmon is attributable to chemical contaminants, early life-stage Chinook salmon will be exposed to water samples collected from selected locations in the Sacramento River, San Joaquin River and Freshwater Delta. Samples will be selected on the basis of chemical analyses and bioassays (*i.e.*, tasks 2.1 and 2.2) to represent locations where high concentrations of (xeno)estrogens are present. Background sites and locations of which low concentrations of (xeno)estrogens are present also will be included. Sediment samples, from one or two locations in which elevated concentrations of sediment-associated contaminants, such as DDE or hydroxylated PCBs are present, also will be analyzed if

the *in vivo* bioassay can be adapted for use with sediments. Assessment of feminization will be made using expression of whole body vitellogenin.

#### *Task 2.4: Analyze extent of feminization with other fish species in the watershed*

In addition to salmon, other fish species may be affected by (xeno)estrogens. In particular, fish that spend a larger fraction of their lives in the Bay/Delta system may be exposed to higher concentrations of (xeno)estrogens than salmon. Most of the proposed research is focused on salmon because they are known to be sensitive to (xeno)estrogens and preliminary data indicate a relatively high frequency of feminized male salmon in the Sacramento and San Joaquin watersheds. However, other fish species may be impacted by (xeno)estrogens and at present no information is available on the extent of feminization of other key species of the Bay/Delta system.

To address the extent that xenoestrogens may be affecting fish that spend a great deal of their lifecycle in the watershed, particularly the estuarine portions that are not covered well by the other tasks, we will sample male fish of several species during their reproductive period. Blood plasma of male fish collected from the Delta will be assayed for egg proteins (*i.e.*, choriogenins). Since males do not make these proteins except when exposed to (xeno)estrogens, their presence is a highly specific biomarker for (xeno)estrogens. The information provided by this task will provide a means for assessing whether or not (xeno)estrogens are acting as a stressor for other fish species that are relevant to the CALFED program.

### **2.3 Experimental Methods**

The research described in the previous section will be conducted using the experimental methods described in the following sections. We are already using many of these methods as part of ongoing research projects. In cases where new methods will be developed, we will use experimental methods developed by other researchers to guide method development activities.

#### **2.3.1 Sample collection**

As part of the activities described in the previous section, water samples will be collected from locations throughout the Sacramento River, San Joaquin River and Delta. Water samples will be collected by Applied Marine Sciences (AMS) using techniques developed as part of the San Francisco Bay Regional Monitoring Program (see [www.sfei.org/rmp/index.html](http://www.sfei.org/rmp/index.html) for details). The staff of AMS has over a decade of experience collecting water, sediment and biota samples in the Bay/Delta system and is well qualified to coordinate the field sampling elements of this program.

The water samples will be collected using a peristaltic pump fitted with Teflon tubing and a 0.2- $\mu$ m in-line cartridge filter. Whenever possible, samples will be collected by boat from the center of the river. However, during storms and at locations where boat access is difficult, samples will be collected from a bridge or from shore using a sample collection boom. As part of sample collection activities, standard quality assurance/quality control procedures will be followed. The QA/QC plan includes the collection of duplicate samples and field blanks at a frequency of at least 5% and maintenance of sample chain of custody to maintain continuity in sample handling.

Samples will be collected in glass containers and will be refrigerated during transit to UC Berkeley and UC Riverside. For chemical analysis and the *in vitro* bioassays, at least 8-L of sample will be collected from each site in 4-L glass containers, with separate 1-L split samples

for analysis of water quality parameters, such as total suspended solids, conductivity, pH and total organic carbon. For the *in vivo* bioassays, to be conducted during the second phase of the project, 100-L samples will be collected from a limited number of sites in glass carboys or other containers. Field spikes will be included in each set of samples to verify the stability of analytes during sample collection, handling and storage.

### 2.3.2 Chemical Analyses

In the first phase of the project, (xeno)estrogens will be analyzed using established methods. In particular, we will target those compounds hypothesized to be important to feminization of salmon (*e.g.*, Table 2). However, we also will include other (xeno)estrogens that can be analyzed readily with the selected analytical techniques. The final selection of compounds to be analyzed will depend upon the method validation studies that will occur during the first three months of the project in which matrix spike recoveries and detection limits will be quantified using the collection methods, handling procedures and sample volumes to be used for the field study.

Solid-phase extraction (SPE) is the first step in the analysis of chemical contaminants and the preparation of samples for the *in vitro* bioassays. To concentrate (xeno)estrogens, we will use pressurized solid-phase extraction on C-18 discs followed by elution with methanol (Kolodziej *et al.* 2003). All of the known (xeno)estrogens listed in Table 2 are extracted efficiently on this material. However, to assure that polar (xeno)estrogens that are not extracted by C-18 are not responsible for the activity, 100-mL aliquots collected before and after C-18 extraction will be subject to SPE with more polar resins (*e.g.*, cation exchangers) or will be subject to lyophilization prior to analysis with the YES assay.

The concentrated C-18 SPE extracts will be analyzed by GC/MS/MS after derivitization to enhance the volatility of the polar compounds. All of the compounds to be analyzed during the first phase of the project can be analyzed by GC/MS/MS (Kolodziej *et al.* 2003, Gross *et al.* 2004, Hoai *et al.* 2003, Schettgen *et al.*, 2002) after the appropriate derivitization techniques are applied. Although most of these compounds also can be analyzed by high-performance liquid chromatography/tandem mass spectrometry (HPLC/MS/MS), we will use GC/MS/MS because it is sensitive and relatively free from interference attributable to organic matter that is present in the SPE extracts.

During the second phase of the project, we will conduct a TIE study to assess the sources of YES and *in vivo* vitellogenin activity using an approach similar to that used by Desbrow *et al.* (1998) and Sapozhikova *et al.* (2005) to identify sources of (xeno)estrogens in municipal wastewater effluent. The initial separation of (xeno)estrogens will be accomplished by selective elution of the C-18 discs with 50-mL aliquots of methanol/water mixtures of decreasing polarity (*e.g.*, 10% methanol, 20% methanol, etc.) followed by measurement of YES and *in vivo* activity in each fraction. Fractions exhibiting activity will be subjected to finer separation by HPLC followed by fraction collection, as described in Huang and Sedlak (2001) and Sapozhikova *et al.* (2005) followed the YES and *in vivo* vitellogenin assays and GC/MS/MS analysis. For extracts in which *in vitro* or *in vivo* activity is greater than that predicted by the GC/MS/MS analyses, extracts will be subjected to analysis by HPLC/MS/MS by Dr. Shane Snyder of the Southern Nevada Water Authority. As part of this activity, we will identify unknown (xeno)estrogens by matching the mass spectra of compounds detected in the extracts with putative compounds that elute at the HPLC retention time associated with each active fraction.

To assure the quality of results from the chemical analyses, appropriate quality assurance/quality control measures will be taken. These steps, which are routine parts of all trace organic analyses conducted in our laboratory, will include analysis of blanks, duplicates and matrix spike recovery samples at a frequency of at least 10%. In addition, each method will include surrogate and internal standards to monitor losses in individual steps in the analysis. After collection of the data, samples that do not meet QA/QC criteria will be eliminated from the database or will be analyzed again if sufficient sample extract remains.

### **2.3.3 YES Assay**

To quantify the concentration of estrogen agonists, we will use the YES *in vitro* assay on sub-samples of the extracts obtained for the chemical analysis. The YES assay, which was developed by Routledge and Sumpter (1996), quantifies all of the compounds in a sample that are capable of binding to an estrogen receptor. The response of the bioassay is compared to a standard curve obtained with 17 $\beta$ -estradiol and the results are expressed as estradiol equivalents (EEQ). The YES assay can be used with an extract from a water sample, provided that the solvent does not interfere with the receptor. A sample volume of only 100  $\mu$ L of extract is needed to detect an EEQ of 1 ng/L in a water sample if a pre-concentration factor of 4,000 is applied. Therefore, the YES assay will require less than 10% of the sample extract from the chemical analysis.

### **2.3.4 *In vivo* Bioassays**

The rainbow trout model will also be used to test *in vivo* estrogenic activity of samples. Juvenile animals of approximately 3 weeks of age will be obtained from the Thomas Fish Company (Anderson CA). The fish will be maintained under flow-through conditions at 10°C if injection exposure is utilized. If static exposure is utilized, 1 mL of extract will be added to 4L of filtered water containing 1 fish. The water will be renewed daily for 7 days. After exposure, animals will be euthanized in MS-222 and bled from the caudal vein using a heparinized syringe. Plasma/serum will be separated by centrifugation and evaluated by commercially available ELISA kits (Biosense, Bergen Norway). Inter-individual variability indicates an N of approximately 10 animals is necessary per exposure.

Data will be evaluated for homogeneity of variance to dictate whether parametric or nonparametric comparisons should be made between fractions or extracts. Appropriate post-hoc tests will be used to determine significance values.

Exposure route significantly affects uptake of xenobiotics into aquatic organisms. One caveat of the research is that the *in vivo* assays will evaluate extracts using aqueous and intraperitoneal injection exposure routes. The later route attempts to mimic oral exposure as the intraperitoneal cavity is drained by the hepatic-portal system allowing uptake to the liver which is the major organ for xenobiotic biotransformation. Unfortunately, this exposure route omits any biotransformation at the gut/intestine that may also contribute to the overall metabolism of any given compound. In addition, environmental bioavailability is also not considered with the injections, as uptake across the gut or gill is bypassed. Overall, the vitellogenin response is a somewhat acute response (7 days) and would not likely identify xenoestrogens that require magnification to levels that may lead to ER activation or indirect estrogenic activity (i.e., anti-androgenic activity). Thus, certain compounds may be excluded using this exposure regime. Pilot studies can be carried out using extended exposure regimes (i.e., 21-30 d) to determine whether differences exist within extracts for activity.

### 2.3.5 *Choriogenin Assay*

The choriogenin assay will be conducted on fish caught during the monthly trawls conducted by California Department of Fish and Game for the Interagency Ecological Program (IEP). Blood will be drawn from the caudal vein or gills of male fish caught in the trawl. Blood will be spun down and the plasma removed and frozen. Several species will be targeted (e.g., Delta smelt, tule perch), but sampling must necessarily remain adaptive, as the abundance of species in the trawls is not predictable. Plasma samples will be analyzed using a choriogenin polyclonal antibody in an ELISA. This antibody strongly cross reacts with choriogenins in a variety of fish species. One of the reasons that the choriogenin assay is preferred over assays for vitellogenin is that the protein structure of this egg component is more highly conserved and thus more widely cross-reactive with antibodies developed in other species. It is expected that both widely ranging species, such as Delta smelt, which will integrate biochemical responses to (xeno)estrogens over a broad area of the watershed, and species that range little within the watershed, such as tule perch will be sampled. Both of these species have been in decline so the data from this task will not only help identify the potential influence of (xeno)estrogens on salmonids in this portion of the estuary, but will be of broader interest to the CALFED program.

## 2.4 Interactions with CALFED Stakeholders and Scientists

One of the major goals of CALFED's science program is to assure that sponsored scientific research has an impact on water management practices in the Bay/Delta system. To assure that the results of this project achieve this goal, we will interact with scientists, managers and stakeholders throughout the project by employing the approach described below.

### 2.4.1 External Advisory Panel

To obtain input on the experimental design and interpretation of results, we will hold regular meetings with a panel of technical advisors with expertise in issues related to salmon and chemical contaminants in the Bay/Delta system (Table 3). The advisors will represent the perspectives of regulators, scientists and managers striving to protect and restore salmon in California's inland waters. Robert Holmes has helped to lead the Central Valley RWQCB's efforts to respond to threats posed by (xeno)estrogens. Dr. Bruce Macfarlane has conducted research on sediment-associated contaminants in the Delta and their effects on salmon. Dr. Thomas Harter is an expert on agricultural wastes and their effects on surface and groundwater systems. All of the advisors listed in Table 3 have expressed a willingness to participate in the project.

Interactions with the external advisory panel will be facilitated through face-to-face meetings and occasional telephone conferences and e-mail correspondence. An initial meeting will be held with the advisory panel during the first month of the project to discuss details of the experimental plan. A second meeting will be held near the completion of Phase I to discuss experimental design for the second phase of the project. A final meeting will be held two months prior to completion of the project to discuss the implications of the research and mechanisms of communicating the results to stakeholders and scientists.

**Table 3:** External advisory panel.

Name	Affiliation
Robert Holmes	Central Valley Regional Water Quality Control Board
Dr. Bruce Macfarlane	NOAA
Dr. Thomas Harter	UC Davis

### 2.4.2 Interactions with other CALFED Projects

Several ongoing CALFED projects are addressing issues that may be important to the experimental design and interpretation of results from this project. For example, Dr. Donald Weston, of UC Berkeley, is leading a CALFED project on the fate of pyrethroid pesticides in the Central Valley and the preliminary findings of his research will be useful to our assessment of the potential for pyrethroids and their degradation products to cause feminization. The current call for proposals emphasizes research on salmonids and we hope to establish strong linkages with other CALFED projects focused on salmonids. Relevant CALFED-funded projects will be identified through discussions with CALFED staff as well as other scientists and stakeholders after initiation of the project.

To assure that results from those ongoing studies are considered in this project, we will make special efforts to facilitate interactions with other researchers through informal contacts between researchers and through formal meetings. For example, one mechanism for facilitating these interactions will involve inviting principal investigators and lead scientists on other

CALFED projects to visit UC Berkeley or UC Riverside and present their recent results at departmental seminars. This approach also will give other CALFED-funded projects an opportunity to receive feedback from outside scientists. After presenting their latest findings, the invited scientists will spend the remainder of day with project personnel exchanging ideas and providing feedback on the experimental design.

### **2.4.3 Dissemination of Results**

Results of our research will be disseminated to the research community through a variety of mechanisms. During the interim stages of the research, results will be presented at regional conferences, such as the biannual CALFED science conference and the California Regional meeting of the Society for Environmental Toxicology and Chemistry (SETAC). We also will develop and maintain a web site on the project on the server for UC Berkeley's Department of Civil and Environmental Engineering. The web site will be linked to home pages for each of the participating investigators and their research groups.

After completion of the different stages of the research, we will publish our results in peer-reviewed scientific journals, such as *Environmental Science and Technology*, *Environmental Toxicology and Chemistry* and *San Francisco Estuary and Watershed Science* (the newly established, open access journal focused on issues of relevance to CALFED). Results also will be presented at national and international scientific meetings. In addition, the lead investigators of this project are often invited to give keynote presentations at highly visible scientific forums and these should provide additional venues to communicate results of the research.

### **2.4.4 Management Plan**

The proposed research is a collaborative effort involving environmental chemists and environmental toxicologists as part of an integrated project focused on the same goal: identifying the causes of feminization of salmon. As a result, all of the researchers will employ the same conceptual model. The boundaries of the research are clearly delineated (*i.e.*, Professor Sedlak's research group will address the chemistry while Professor Schlenk's group will be responsible for the toxicology) despite the fact that the research is fully integrated. Because chemical analyses will be performed on the same water samples in which *in vitro* and *in vivo* bioassays will be conducted, the interpretation of data is interdependent. This integrated experimental design is particularly powerful because it offers independent lines of evidence for findings from the two approaches.

Although analyses will be conducted on the same water samples, the schedule of the individual groups will not be hindered by minor setbacks or delays experienced by one of the groups because samples will be collected by a third party (*i.e.*, AMS). Furthermore, samples undergoing chemical analysis and *in vitro* bioassays will be extracted and split immediately after collection, thereby preventing any delays associated with analyses.

Professors Sedlak and Schlenk have successfully collaborated on other research projects. They are currently collaborating on a research project funded by the University of California's Coastal Marine Science Program, investigating the causes of feminization of flatfish in coastal waters. In that project, Professor Schlenk was responsible for bioassays while Professor Sedlak and Dr. Kolodziej, who was a doctoral student during the project, were responsible for chemical analyses of sediment samples. In addition, Professor Schlenk has served as an informal advisor to two of Professor Sedlak's doctoral students, who studied the fate of steroid hormones in an

engineered wetland in Southern California. As a result of these previous interactions, the lines of communication between the two research groups are well established.

Throughout the research project, effective communication between the two groups will be reinforced through several different mechanisms. The main mechanism to ensure effective communication is through face-to-face contact. Full-day meetings of the lead investigators and key personnel will occur in Berkeley or Riverside at the start of the first and second phases of the project. In addition, meetings will take place among staff involved in sample collection and analysis at the beginning of the period of intensive field sampling (*i.e.*, late fall). Furthermore, progress report meetings will be held by conference call every two months to discuss progress, scheduling and any impediments in the research program.

In addition to the group meetings and conference calls, the project manager, Dr. Edward Kolodziej, will dedicate approximately 20% of his time to project oversight and management. Dr. Kolodziej will be responsible for coordinating meetings, evaluating project schedules and timelines and facilitating communication between researchers. To accomplish these objectives, Dr. Kolodziej will visit UC Riverside once every two months to meet with Dr. Schlenk's research team. In addition, Dr. Kolodziej, will accompany AMS during the first rounds of sampling during the first and second phases of the research project. Dr. Kolodziej is particularly well suited for this task (see CV for details) because he is familiar with many of the proposed sampling locations has a strong background in environmental chemistry and toxicology and already has worked closely with Professors Sedlak and Schlenk.

### **3. Justification**

Previous research in our laboratories and by scientists working in the Sacramento and San Joaquin watersheds and other locations suggests that feminization of Chinook salmon is occurring and that the most likely cause is exposure to (xeno)estrogens. Feminization is believed to have important impacts on the growth, survival and reproduction of many fish species. In species like Chinook salmon, that are sensitive to chemical stresses, feminization may be an important but overlooked stressor that contributes to the population declines that have been observed among salmonids in the Sacramento and San Joaquin ecosystems. Presently, very little is known about the causes of feminization of salmonids. The research described in this proposal will determine the chemical causes of feminization in Chinook salmon, will determine the sources of (xeno)estrogens in the Sacramento and San Joaquin watersheds, and will develop toxicological models for the effects of (xeno)estrogens on Chinook salmon. The research also will provide much needed data on the frequency of feminization of other key species that are resident to the Delta. Finally, the research also aims to identify cost-effective methods to control or alleviate the impacts of (xeno)estrogens on sensitive fish populations in these watersheds and the greater Bay-Delta.

This research project addresses several issues of importance to the CALFED program. In particular, the research is relevant to efforts to protect and restore Chinook salmon and other critical species because it provides much needed information about a family of chemical stressors that have not received much attention from the CALFED program. Many of the contaminants that are hypothesized to be responsible for feminization of fish are released from sources that exist in the Bay/Delta system. However, scientific information is not available to compare the relative importance of (xeno)estrogens and other fish stressors. If (xeno)estrogens prove to be important stressors, our research will help CALFED to design future monitoring programs and control measures for these stressors.

If feminization of salmon is caused by chemical contaminants present in water flowing through the Delta, efforts to enhance salmonids and other sensitive fish species by alteration of water flows and reallocations of water resources could be compromised because the concentrations of (xeno)estrogens or the timing of these discharges may be inadvertently altered by changing the relative flows from different sources of water. Furthermore, water diversions or changes in agricultural practices that alter the retention times of (xeno)estrogens in environments where they might be removed through natural attenuation could result in exposure of fish to higher concentrations of contaminants. These issues are particularly important in the Delta where planned alterations in flows could result in changes in the composition and timing of water discharges.

#### 4. Literature Cited

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- Williamson, K.S., May, B. 2002. Incidence of phenotypic female Chinook salmon positive for the male Y-chromosome-specific marker *OtY1* in the Central Valley, California. *J. Aquat. Anim. Health.* 14:176-183.

## DAVID L. SEDLAK

### EDUCATION

**University of Wisconsin**, Madison, Wisconsin  
Water Chemistry  
**Dissertation:** Abiotic Oxidation of Polychlorinated Biphenyls (PCBs)  
Ph.D.  
June 1992

**Cornell University**, Ithaca, New York  
Environmental Science  
B.S.  
June 1986

### EXPERIENCE

October 1994-Present: **Professor (2004-present), Associate Professor (2000-2004) and Assistant Professor (1994-2000)**, Department of Civil and Environmental Engineering, University of California, Berkeley, CA

July 2003-June 2004: **Visiting Associate Professor**, School of Civil and Environmental Engineering, University of New South Wales, Sydney, Australia

April 1992-June 1994: **Postdoctoral Fellow**, Swiss Federal Institute for Environmental Science and Technology, Dübendorf, Switzerland

July 1986-June 1988: **Staff Scientist**, ENVIRON Corporation, Princeton, New Jersey

### RESEARCH INTERESTS

Fate of wastewater-derived chemical contaminants in conventional and advanced wastewater treatment plants, fate of steroid hormones in the aquatic environment, chemical fate during groundwater recharge and in engineered treatment wetlands, metal speciation in soil and water.

### AWARDS

Fulbright Senior Scholar Award for Australia, 2003  
Paul L. Busch Award for Innovation in Applied Water Quality Research, 2003  
National Science Foundation CAREER Development Award, 1998  
Hellman Family Fund Faculty Award, 1995  
Graduate Student Award, ACS Division of Environmental Chemistry, 1991  
Graduate Student Paper Award, ACS Division of Environmental Chemistry, 1990  
Carl Ladd Scholarship, Cornell University, 1985  
New York State Regents Scholarship, Cornell University, 1982-1986

### PROFESSIONAL AFFILIATIONS AND SERVICE

Chair, Gordon Research Conference Environmental Sciences Water, 2004  
Editorial Board Member, Environmental Technology  
Member, US EPA Science Advisory Board, Drinking Water Committee  
Member, American Chemical Society  
Member, Association of Environmental Engineering Professors  
Member, California Water Environment Association  
Member, Society of Environmental Toxicology and Chemistry

Member, International Water Association  
Reviewer for Scientific Journals (approximately 75 reviews in past 5 years)  
Review Panel Member, National Science Foundation (1998, 2002, 2003)

#### **UNIVERSITY SERVICE**

Group Leader, Environmental Engineering Program  
Member, Committee on Undergraduate Prizes  
Member, Committee on Undergraduate Scholarships and Honors (2000-2003)  
Member, Environmental Sciences Advisory Board, College of Natural Resources

#### **SPONSORED RESEARCH PROJECTS (CURRENT)**

- “The Environmental Fate of Effluent-Derived Chemical Contaminants.” Sponsored by the National Water Research Institute (duration: 3/00-9/04).
- “Removal of NDMA and NDMA Precursors During Wastewater Treatment.” (w/M. Kavanaugh, Co-PI) Sponsored by the WateReuse Foundation. (duration 4/02-9/04).
- “Identification of Estrogenic Compounds in California Coastal Waters.” (w/D. Schlenck, R. Tjeerdma, Co-PIs). Sponsored by the University of California Marine Council. (duration 7/02-11/04).
- “Fate of NDMA and NDMA Precursors in the Aquatic Environment” (Lead PI with L. Alvarez-Cohen, P. Fox, J. Gan) Sponsored by the WateReuse Foundation (duration 4/03-4/05).
- “Quantification of Unintentional Water Recycling in Surface Waters.” (w/J. Dracup, co-PI). Sponsored by the National Science Foundation. (duration 4/03-3/05).

#### **CURRENT DOCTORAL STUDENTS AND TOPICS**

Timothy Durbin-Fate of NDMA during groundwater recharge  
Lorien Fono-Removal of chiral pharmaceuticals in engineered wetlands  
Mong-Hoo Lim-Fate of wastewater-derived contaminants in advanced treatment systems  
Elif Pehlivanoglu-Environmental fate of effluent-derived organic nitrogen

#### **PEER-REVIEWED PUBLICATIONS**

- Gray J.L. and Sedlak D.L. (2005) The fate of estrogenic hormones in an engineered treatment wetland with dense macrophytes. *Water Environ. Res.*, In Press.
- Sedlak D.L., Deeb R.A., Hawley E.L., Mitch W.A., Durbin T.D., Mowbray S., Carr S. (2005) Sources and fate of nitrosodimethylamine and its precursors in municipal wastewater treatment plants. *Water Environ. Res.*, In Press.
- Joo S.H., Feitz A.J., Sedlak D.L. and Waite T.D. (2005) Quantification of the oxidizing capacity of nanoparticulate zero-valent iron. *Environ. Sci. Technol.*, In Press.
- Kolodziej E.P., Harter T. and Sedlak D.L. (2004) Dairy wastewater, aquaculture and spawning fish as sources of steroid hormones in the aquatic environment. *Environ. Sci. Technol.*, 38, 6377-6384.
- Sedlak D.L., Huang C.H. and Pinkston K.E. (2004) Strategies for selecting pharmaceuticals to assess attenuation during indirect potable water reuse. In: *Pharmaceuticals in the environment*. K. Kümmerer, ed. Springer Publishers, Berlin.
- Pinkston K.E. and Sedlak D.L. (2004) Transformation of Aromatic Ether- and Amine-Containing Pharmaceuticals during Chlorine Disinfection. *Environ. Sci. Technol.*, 38, 4019-4025.
- Mitch W.A. and Sedlak D.L. (2004) Characterization and fate of NDMA precursors in municipal wastewater treatment plants. *Environ. Sci. Technol.*, 38, 1445-1454.

- Ridge A.C. and Sedlak D.L. (2004) Effect of ferric chloride addition on the removal of Cu and Zn complexes with EDTA during municipal wastewater treatment. *Water Research*, 38, 921-934.
- Kolodziej E.P., Gray J.L. and Sedlak D.L. (2003) Quantification of steroid hormones with pheromonal properties in municipal wastewater effluent. *Environmental Toxicology and Chemistry*. 22, 2622-2629.
- Mitch W.A., Sharp J.O., Trussell R.R., Valentine R.L., Alvarez-Cohen L. and Sedlak D.L. (2003) *N*-Nitrosodimethylamine as a drinking water contaminant: A review. *Environmental Engineering Science* 20, 389-404.
- Snyder S.A., Westerhoff P., Yoon Y. and Sedlak D.L. (2003) Pharmaceuticals, personal care products and endocrine disrupters in water: Implications for water treatment. *Environmental Engineering Science* 20, 449-469.
- Mitch W.A., Gerecke A.C. and Sedlak D.L. (2003) A *N*-Nitrosodimethylamine (NDMA) precursor analysis for chlorination of water and wastewater. *Water Research* 37, 3733-3741.
- Mehrotra A.S., Horne A.J. and Sedlak D.L. (2003) Inhibition of net mercury methylation by iron in *Desulfobulbus propionicus* cultures: implications for engineered wetlands. *Environ. Sci. Technol.* 37, 3018-3023.
- Hsu H. and Sedlak D.L. (2003) Strong Hg(II) complexation in municipal wastewater effluent and surface waters. *Environ. Sci. Technol.* 37, 2743-2749.
- Gerecke A.C. and Sedlak D.L. (2003) Precursors of *N*-Nitrosodimethylamine (NDMA) in natural waters. *Environ. Sci. Technol.* 37, 1331-1336.
- Mitch W.A. and Sedlak D.L. (2002) Factors affecting the formation of NDMA during chlorination. *Environ. Sci. Technol.*, 36, 588-595.
- Bedsworth W.W. and Sedlak D.L. (2001) Determination of metal complexes of ethylenediaminetetraacetate (EDTA) in the presence of organic matter by high performance liquid chromatography. *J. Chromatography A*, 905, 157-162.
- Huang, C.H. and Sedlak, D.L. (2001) Analysis of estrogenic hormones in municipal wastewater effluent and surface water using ELISA and GC/MS/MS. *Environmental Toxicology and Chemistry*. 20, 133-139.
- Sedlak D.L., Gray J.L. and Pinkston K.E. (2000) Understanding microcontaminants in recycled water. *Environ. Sci. Technol.* 34, 508A-515A.
- Weissmahr K.W. and Sedlak D.L. (2000) Influence of metal complexation on the degradation of dithiocarbamate fungicides. *Environ. Toxicol. & Chem.* 19, 820-826.
- Voelker B., Sedlak D.L. and Zafiriou O.C. (2000) Chemistry of superoxide radical in seawater: reactions with organic Cu complexes. *Environ. Sci Technol.* 34,1036-1042.
- Abu-Saba K., Flegal A.R. and Sedlak D.L. (2000) Reduction of hexavalent chromium by copper in the presence of superoxide. *Marine Chemistry*. 69, 33-41.
- Bedsworth W.W. and Sedlak D.L. (1999) Sources and environmental fate of strongly complexed nickel in estuarine waters: the role of ethylenediaminetetraacetate. *Environ. Sci. Technol.* 33(6): 926-931.
- Zafiriou O.C., Voelker B. and Sedlak D.L. (1998) Chemistry of superoxide radical in seawater: reactions with inorganic Cu complexes. *J. Phys. Chem. A* 102, 5693-5700.
- Weissmahr K.W., Houghton C.L. and Sedlak D.L. (1998) Analysis of the dithiocarbamate fungicides Ziram, Maneb, Zineb and the flotation agent Ethylxanthogenate by ion-pair reversed phase HPLC. *Anal. Chem.* 70, 4800-4804.

- Sedlak D.L., Phinney J.T. and Bedsworth W.W. (1997) Strongly complexed Cu and Ni in wastewaters and surface runoff. *Environ. Sci. Technol.* 31(10), 3010-3016.
- Sedlak D.L. and Chan P.G. (1997) The reduction of Cr(VI) by Fe(II) in natural waters. *Geochimica et Cosmochimica Acta*, 61, 2185-2192.
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- Voelker, B. and Sedlak, D.L. (1995) Iron reduction by photoproducted superoxide in seawater. *Marine Chemistry* 50, 93-102.
- Matthijsen J., Builtjesi P.J.H. and Sedlak D.L. (1995) Cloud model experiments on the effect of iron and copper on tropospheric ozone under marine and continental conditions. *Meteorology and Atmospheric Physics*, 57, 43-60.
- Sedlak D.L. and Andren A.W. (1994) The effect of sorption on the oxidation of polychlorinated biphenyls (PCBs) by hydroxyl radical. *Water Res.* 28(5), 1207-1215.
- Sedlak D.L. and Hoigné J. (1994) The oxidation of S(IV) in atmospheric waters by photo-oxidants and iron in the presence of copper. *Environ. Sci. Technol.* 28(11), 1898-1906.
- Sedlak D.L. and Hoigné J. (1993) The role of copper and oxalate in the redox cycling of iron in atmospheric waters. *Atmospheric Environment* 27A(14), 2173-2185.
- Sedlak D.L., Dean K.E., Armstrong D.E. and Andren A.W. (1991) Interaction of quicklime with polychlorobiphenyl-contaminated solids. *Environ. Sci. Technol.* 25, 1936-1940.
- Sedlak D.L. and Andren A.W. (1991) Aqueous-phase oxidation of polychlorinated biphenyls with hydroxyl radicals. *Environ. Sci. Technol.* 25(8), 1419-1427.
- Sedlak D.L. and Andren A.W. (1991) Oxidation of chlorobenzene with Fenton's reagent. *Environ. Sci. Technol.* 25(4), 777-782.

#### **CONFERENCE TALKS AND INVITED PRESENTATIONS (PARTIAL LIST)**

- “Understanding Microcontaminants in Recycled Water”, Keynote presentation, OZ-AQUAREC Workshop IV, Detection, Fate and Removal of Trace Contaminants, Wollongong, Australia, February 13, 2004.
- “The Fate of Wastewater-Derived Contaminants in Engineered and Natural Systems”, Departmental Seminar, CSIRO, Adelaide, Australia, January 29, 2004.
- “Quantification of NDMA Precursors in the Aquatic Environment”, American Water Works Association 2003 National Meeting, Anaheim, CA, June 18, 2003.
- “Wastewater-Derived Chemical Contaminants to Water Providers”, American Water Works Association 2003 National Meeting, Anaheim, CA, June 18, 2003.
- “Occurrence and Treatment of Endocrine Disrupters and Pharmaceuticals in Municipal Wastewater Effluent”, San Gabriel Watermaster, Azusa, CA, May 29, 2003.
- “The Fate of Wastewater-Derived Contaminants in Engineered Treatment Wetlands”, Departmental Seminar, Department of Oceanography, Stony Brook University, Stony Brook, NY, May 2, 2003.
- “Wastewater-Derived Chemical Contaminants to Water Providers”, American Water Works Association California and Nevada Regional Meeting, Reno, NV, October 16, 2002.
- “Wastewater-Derived Chemical Contaminants.” Association of California Water Agencies Conference on Xenobiotics. Sacramento, CA, September 2002.

- "N-Nitrosodimethylamine: The Unexpected Disinfection Byproduct." Gordon Research Conference, Environmental Sciences: Water. Plymouth, NH, June 2002.
- "Emerging Contaminants: New Research Opportunities for Bioremediation Specialists." NIEHS Conference on Bioremediation, Monterey, CA. June 2002.
- "Factors Controlling the formation of N-Nitrosodimethylamine (NDMA) during Chlorination." Water Reuse Foundations, 2001 Annual Research Conference. Monterey, CA.
- "Factors Affecting the Fate of Pharmaceuticals in the Aquatic Environment." National Ground Water Association International Conference on Pharmaceuticals and Endocrine Disrupters. Minneapolis, MN, November 2001.
- "Emerging Issues in Environmental Chemistry." Department of Environmental Toxicology and Chemistry, Oregon State University, Corvallis, OR. November 2001.
- "Effluent-Derived Chemical Contaminants in Recycled Water." Department of Civil and Environmental Engineering, MIT, Boston, MA. November 2001.
- "Endocrine Disrupters in Municipal Wastewater." Department of Environmental Engineering, National Autonomous University of Mexico (UNAM), Mexico City, Mexico. July 2001.
- "Challenges Associated with Quantification of Trace Concentrations of Pharmaceutically-Active Compounds (PhACs) in a Complex Matrix." American Water Works Association Research Foundation Emerging Contaminants Conference, Chicago, IL. April 2001.
- "The Fate and Transport of Hormones in the Aquatic Environment." Environmental Engineering Science seminar series, California Institute of Technology, Pasadena, CA. March 2001.
- "Analytical Challenges Associated with Identification of Endocrine Disrupters in Water." American Water Works Association Special Symposium, Denver, CO. March 2001.
- "Immunochemical Methods for Quantifying Hormones in Polluted Waters." Swiss Chemical Society Meeting, Basel, Switzerland. November 2000.
- "The Environmental Chemistry of Water Reuse." Harvard University College of Engineering and Applied Sciences, May 1999.
- "Pharmaceutically Active Compounds (PhACs) in the Aquatic Environment and their Relationship to Water Reuse." Plenary lecture, 9th Biennial Symposium on Artificial Recharge of Groundwater, Phoenix, AZ, June 1999.
- "The Role of Speciation in the Removal of Cationic Metals by Wastewater Treatment Systems: A Short Course on Metal Removal in Wastewater Treatment Plants." Water Environment Research Foundation, Orlando, FL, October 98.
- "Metals as Catalysts of Sunlight-Induced Reactions in Natural Waters." Geological Society of America, 1998 Annual Meeting, Toronto, Canada, October 1998.
- "Analytical Techniques for Determining Metal Speciation in Polluted Waters." Plenary lecture, Fifth International Argentum Conference, Hamilton, Ontario, Canada, September 1997.
- "Thermodynamic Data and the Prediction of Metal Speciation in Polluted Waters." National Institute of Standards and Technology (NIST), Gaithersburg, MD, August 1998.
- "The Treatment and Environmental Fate of Strongly Complexed Metals." Department of Civil and Environmental Engineering, UC Davis, November 1997 and Department of Civil Engineering, University of Nevada, Reno, February 1998.
- "Superoxide radical ( $O_2^-$ ) and the Photoredox Chemistry of Copper and Chromium." 18th Annual Meeting of the Society of Environmental Toxicology and Chemistry, San Francisco, CA, November 1997.
- "Analytical Techniques for Determining Metal Speciation in Polluted Waters." Plenary lecture, Fifth International Argentum Conference, Hamilton, Ontario, Canada, September 1997.

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**Education**

Ph.D. Biochemical Toxicology; Oregon State University, June 1989

B.S. Toxicology; Northeast Louisiana University, May 1984

**Employment**

2000-now Professor Aquatic Ecotoxicology, Department of Environmental Sciences, University of California, Riverside

1999-2000 Program Coordinator of Environmental Toxicology Program, Environmental and Community Health Research Program, School of Pharmacy, University of Mississippi

1998-2000 Associate Professor of Pharmacology and Toxicology, University of Mississippi

1995-1998 Assistant Professor of Pharmacology and Toxicology University of Mississippi

1991-1995 Assistant Professor Toxicology, University of Arkansas for Medical Sciences

1989-1991 Postdoctoral Fellow, Duke University

1986-1989 Predoctoral Fellow, Oregon State University,

**Professional Activities**

- Editorial Board (2003- present), *Environmental Toxicology and Chemistry*
- Editorial Board (2001- present), *Aquatic Toxicology*
- Editorial Board (2000- present), *Marine Environmental Research*
- Editorial Board (2000- present), *Toxicological Sciences*
- Member (since 1991), Society of Toxicology
- Member (since 1993), Society of Environmental Toxicology and Chemistry
- Member (since 1999), International Society of the Study of Xenobiotics

**Academic Honors**

- Visiting Scholar, Department of Biochemistry; Chinese University of Hong Kong 1995; 1998; 1999
- Ray Lankester Investigatorship -Marine Biological Association of the United Kingdom 1998
- Visiting Scholar, Instituto Del Mare, Venice Italy 1999
- University of Mississippi; School of Pharmacy, Faculty Research Award, 1999-2000
- Member of Eleventh International Pollution Responses in Marine Organisms Symposium Scientific Advisory Committee, Plymouth, United Kingdom 2001
- George E. Brown, Jr. Award (UCMEXUS) Co-PI with J. Garcia-Hernandez 2001
- Visiting Scholar, CSIRO Laboratory Lucas Heights, Australia 2003

- Elected to the Society of Environmental Toxicology and Chemistry North American Board of Directors 2003-2006
- Invited Opponent for Doctoral Dissertation, University of Gothenburg, Sweden

### **Edited Books**

D. Schlenk and W.H. Benson (2001) *Target Organ Toxicity in Marine and Freshwater Teleosts* Volume 1. Taylor and Francis Publishers, Washington DC.

D. Schlenk and W.H. Benson (2001) *Target Organ Toxicity in Marine and Freshwater Teleosts* Volume 2. Taylor and Francis Publishers, Washington DC.

### **Book Chapters (7 Total)**

Y. Sapozhnikova, A. Mcelroy, S. Snyder and D. Schlenk (2005) Estrogenic activity measurement in wastewater using in vitro and in vivo methods In: *Methods of Aquatic Toxicology* Lewis Publishers; Boca Raton, FL. (in press).

### **Selected Peer-Reviewed Publications (105 Total)**

D. Schlenk, D. Stresser, A. Nimrod, L. Arcand and W.H. Benson (1997) Influence of B-naphthoflavone and methoxychlor pretreatment on the biotransformation and estrogenic activity of methoxychlor in channel catfish (*Ictalurus punctatus*). *Toxicology and Applied Pharmacology* 145:349-356.

D. Schlenk, D. M. Stresser, J. Rimoldi, L. Arcand, J. McCants, A.C. Nimrod, and W.H. Benson (1998) Biotransformation and estrogenic activity of methoxychlor and its metabolites in channel catfish (*Ictalurus punctatus*) *Marine Environmental Research* 46:159-162

D. Schlenk (1999) Necessity of defining biomarkers for use in ecological risk assessments. *Marine Pollution Bulletin* 39:48-53.

M. McArdle, A. Elskus, A. McElroy, B. Larsen, W. Benson, and D. Schlenk. (2000) Differences of estrogenic response in two species, *Fundulus heteroclitus* and *Morone saxatilis*. *Marine Environmental Research* 50:175-179.

S. Thompson, F. Tilton, D. Schlenk, and W.H. Benson. (2000) Comparative vitellogenic response in three teleost species: Extrapolation to in situ field studies. *Marine Environmental Research* 50: 185-189.

E. Perkins, B.C. DeBusk and D. Schlenk (2000) Isolation and characterization of a novel cytochrome P450 (CYP2 family) isoform from channel catfish. *Fish Physiology and Biochemistry* 22:199-206.

D. L. Straus, D. Schlenk, and J. E. Chambers (2000) Hepatic microsomal desulfuration and dearylation of chlorpyrifos and parathion in fingerling channel catfish: lack of effect from aroclor 1254 *Aquatic Toxicology* 50:141-149.

- D. Schlenk, E.J. Perkins, and B.C. DeBusk (2000) 2-Methylisoborneol disposition in three strains of catfish: absence of biotransformation. *Fish Physiology and Biochemistry* 23:225-232.
- A. El-Alfy, S. Grisle, and D. Schlenk (2001) Characterization of Salinity-enhanced toxicity of aldicarb to Japanese medaka: sexual and developmental differences. *Environmental Toxicology and Chemistry* 20:2093-2098.
- F. Tilton, W. H. Benson, and D. Schlenk (2001), Elevation of serum 17- $\beta$ -estradiol in channel catfish following injection of 17- $\beta$ -estradiol, ethynyl estradiol, estrone, estriol and estradiol-17- $\beta$ -glucuronide. *Environmental Toxicology and Pharmacology* 9:169-172
- D. Schlenk, D. Huggett, D.B. Block, D.S., Grisle, S., Allgood, J., Bennet, E., Holder, A.W., Hovinga, R.M. Bedient, P. (2001) Toxicity of Fipronil and its Degradation Products to *Procambarus sp.*: Field and Laboratory Studies. *Archives of Environmental Contamination and Toxicology* 41: 325-332.
- F.X. Han, J.A. Hargreaves, W.L. Kingery, D.B. Huggett, D. Schlenk (2001) Accumulation, distribution, and toxicity of copper in sediments of catfish ponds receiving periodic copper sulfate applications. *Journal of Environmental Quality* 30:912-919.
- Beeler, A. B.; Schlenk, D.; Rimoldi, J. M. Synthesis of fipronil sulfide, an active metabolite, from the parent insecticide fipronil. *Tetrahedron Lett.* (2001), 42(32), 5371-5372.
- J. Wang, S. Grisle, and D. Schlenk (2001) Effects of salinity on aldicarb toxicity to juvenile rainbow trout (*Oncorhynchus mykiss*) and striped bass (*Morone saxatilis x chrysops*). *Toxicological Sciences* 64:200-207.
- I.A. Khan, J. Allgood, L.A. Walker, E.A. Abourashed, D. Schlenk, W.H. Benson (2001) Determination of heavy metals and pesticides in ginseng products. *J. AOAC Int.* 84: 936-939.
- B. Larsen, and D. Schlenk (2002) Effect of Urea and Temperature on the Expression and Activity of Flavin-Containing Monooxygenase expression in the liver and gill of rainbow trout (*Oncorhynchus mykiss*). *Fish Physiology and Biochemistry* 25:19-29.
- D. Schlenk, B. Furnes, X. Zhou, and B.C. Debusk (2002) Cloning and sequencing of cytochrome P450 2X1 from channel catfish (*Ictalurus punctatus*). *Marine Environmental Research* 54:391-394.
- J. R. Todorov, A. A. Elskus, D. Schlenk, P. L. Ferguson, B. J. Brownawell, and A. E. McElroy (2002) Estrogenic Responses of Larval Sunshine Bass (*Morone saxatilis X M. chrysops*) Exposed to New York City Sewage Effluent. *Marine Environmental Research* 54:691-695.
- F. Tilton, W.H. Benson, and D. Schlenk (2002) Evaluation of Estrogenic Activity from a Municipal Wastewater Treatment Plant with Predominantly Domestic Input. *Aquatic Toxicology* 61:211-224.

A. El-alfy, E. Bernache, and D. Schlenk (2002) Effects of salinity on the uptake and elimination of aldicarb in Japanese medaka *Aquatic Toxicology* 61:225-232.

D.Schlenk,, E. Sapozhnikova, J.P. Baquirian, and A.Z.Mason (2002) Utilization of biochemical and health endpoints in fish to guide analytical chemistry analyses of sediments. *Environmental Toxicology and Chemistry* 21: 2138-2145.

A. El-Alfy, B. Larsen, and D. Schlenk (2002) Effect of Cortisol and Urea on Flavin Monooxygenase Activity and Expression in Rainbow Trout, *Oncorhynchus mykiss*. *Marine Environmental Research* 54:275-278.

A. El-Alfy, and D. Schlenk (2002) Effects of 17-beta estradiol and testosterone on the expression of flavin containing monooxygenase mediated toxicity of aldicarb in Japanese medaka. *Toxicological Sciences* 68:381-388.

D.B.Huggett, B.W. Brooks, B. Peterson, C.M. Foran, D. Schlenk. (2002) Toxicity of Select Beta-Adrenergic Receptor Blocking Pharmaceuticals ( $\beta$ -Blockers) on Aquatic Organisms. *Archives of Environmental Contamination and Toxicology* 43:229-235.

D.B. Huggett, I.A. Khan, C.M. Foran and D. Schlenk (2002) Determination of Beta-Adrenergic Receptor Blocking Pharmaceuticals in United States Wastewater Effluent *Environmental Pollution* 121:199-205.

D. Schlenk, X. Zhang, C. Yeung, J. Zhang, J. Cashman, A. Rettie (2002) Role of flavin-containing monooxygenases in the sulfoxidation of aldicarb in humans. *Pesticide Biochemistry and Physiology* 73: 67-73.

R. Riedel, D. Schlenk, D. Frank, B. Costa-Pierce (2002) Analyses of organic and inorganic contaminants in Salton Sea fish. *Marine Pollution Bulletin* 44:403-411.

L. A. Roy, J. L. Armstrong , K. Sakamoto, S. Steinert , E. Perkins' D. P. Lomax , L. L. Johnson and D.Schlenk (2003) The relationships of biochemical endpoints to histopathology, and population metrics in feral flatfish species collected near the municipal outfall of Orange County,CA. *Environmental Toxicology and Chemistry* 22:1309-1317.

B. Furnes, J., Feng, S.,Sommer, and D. Schlenk (2003) Identification of novel variants of the flavin-containing monooxygenase gene family in African Americans. *Drug Metabolism and Disposition* 31:187-193.

D. Schlenk (2003) Use of Biochemical Endpoints to determine relationships between contaminants and impaired fish health in a freshwater stream. *Human and Ecological Risk Assessment* 9:59-66.

Huggett, D.B., C.M. Foran, B. Brooks, J. Weston, B.P. Peterson, E.M. Marsh, D. Schlenk (2003) Comparison of In vitro and in vivo bioassays for estrogenicity in fractionated effluent from Municipal Wastewater Effluents. *Toxicological Sciences* 72:77-83.

V. Lattard, J. Zhang, Q., Tran, B. Furnes, D. Schlenk, J. R. Cashman (2003) Two novel polymorphisms of the FMO3 gene in Caucasians and African American populations: Comparative genetic and functional studies. *Drug Metabolism and Disposition* 31:854-860.

L.A. Roy, S. Steinert, S.M. Bay, D. Greenstein, Y. Sapozhnikova, O. Bawardi, I. Leifer and D. Schlenk (2003) Biochemical effects of PAH exposure in hornyhead turbot (*Pleuronichthys verticalis*) exposed to a gradient of PAH contaminated sediments collected from a natural petroleum seep in CA, USA. *Aquatic Toxicology* 65:159-169.

D. Schlenk, N. Zubcov, E. Zubcov (2003) Effects of salinity on the uptake, biotransformation and toxicity of dietary seleno-L-methionine to rainbow trout. *Toxicological Sciences* 75:309-313.

D. Schlenk, C. Yeung, A. Rettie (2004) Unique Stereoselective Sulfoxidation of Thioethers Indicates Novel Flavin-Containing Monooxygenase in Liver of Rainbow Trout. *Marine Environmental Research* 58: 499-503.

E. Sapozhnikova, O. Bawardi, L. Roy, D. Schlenk, D (2004) Pesticides and PCBs in sediments and fish from the Salton Sea, California, USA. *Chemosphere* 55:797-809.

K. Schrader, C. Foran, C. Peterson, D. Schlenk (2004) Toxicological Evaluation of Two Anthraquinone-based Cyanobactericides towards Channel Catfish *Ictalurus punctatus* North American Journal of Aquaculture 66:119-124.

B. Furnes and D. Schlenk (2004) Evaluation of Xenobiotic N- and S-oxidation by Variant Flavin-containing Monooxygenase 1 (FMO1) Enzymes. *Toxicological Sciences* 78:196-203.

L. Xie, Y. Sapozhnikova, O. Bawardi, and D. Schlenk (2004) Evaluation of wetland and tertiary wastewater treatments for estrogenicity using in vivo and in vitro assays. *Archives of Environmental Contamination and Toxicology* (in press).

B. Furnes and D. Schlenk (2004) Extrahepatic metabolism of carbamate and organophosphate thioether compounds by the FMO and P450 system. *Drug Metabolism and Disposition* (in press).

Y. Sapozhnikova, E. Zubcova, L. Ungureanu, L. Roy, D. Schlenk (2004) Evaluation of pesticides, metals, and metallothionein expression in fish of the Dniester River, Moldova *Chemosphere* (accepted).

D. Vidal, S. Bay, D. Schlenk (2004) Effects of selenium accumulation on larval rainbow trout (*Oncorhynchus mykiss*). *Archives of Environmental Contamination and Toxicology* (accepted).

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## EDUCATION

**UNIVERSITY OF CALIFORNIA AT BERKELEY. Ph.D.**, Environmental Engineering, Fall 2004.  
Dissertation topic: *The Occurrence and Environmental Fate of Steroid Hormones with Endocrine and Pheromonal Activity in Fish*. Minors in Endocrinology and Ecological Chemistry.

**UNIVERSITY OF CALIFORNIA AT BERKELEY. M.S.**, with honors in Environmental Engineering, 1999. GPA: 3.9.

**JOHNS HOPKINS UNIVERSITY. B.S.**, with general honors in Chemical Engineering, 1998. GPA: 3.6

## RESEARCH EXPERIENCE

**UC Berkeley**, Berkeley, CA. August 1999 – December 2004. Ph.D. Candidate in Environmental Engineering.

- Research focused on the occurrence and environmental fate of a suite of steroid hormones, often present in municipal wastewater effluent or agricultural discharges, that also are implicated in endocrine disruption or disruption of pheromone signaling in fish. Responsibilities included experimental design, GC/MS/MS analytical method development, field sampling, chemical analysis, and interpretation, presentation, and publication of experimental data.

**UC Berkeley**, Berkeley, CA. October 1998 - August 1999. Graduate Student Researcher.

- Research focused upon the design and implementation of a novel hydroxyapatite chemical treatment process for remediation of acid mine drainage in California's Lake Shasta region.

**Michigan Technological University**, Houghton, MI. Summer 1997. Student Intern for the Environmental Protection Agency's Science to Achieve Results (STAR) program.

- Research focused upon the formation and control of trihalomethane disinfection by-products in the drinking water supply for New York City.

## TEACHING EXPERIENCE

**UC Berkeley**, Berkeley, CA. Graduate Student Instructor.

- Spring 2002. *Environmental Chemical Kinetics*. Graduate level environmental chemistry lecture. Professor David L. Sedlak. Responsibilities included teaching one lecture/discussion section per week, exam preparation, preparing homework solutions, and weekly office hours.

## PUBLICATIONS

Kolodziej E.P., Gray J.L., and Sedlak D.L. 2003. "Quantification of Steroid Hormones with Pheromonal Properties in Municipal Wastewater Effluent." *Environ. Toxicol. Chem.*, **22**(11), 2622-2629.

Sedlak D.L., Pinkston K.L., Gray J.L. and Kolodziej E.P. 2003. "Approaches for Quantifying the Attenuation of Wastewater-Derived Contaminants in the Aquatic Environment." *Chimia*. **57**(9), 567-569.

Kolodziej E.P., Harter T.H., and Sedlak D.L. 2004. "Dairy Wastewater, Aquaculture, and Spawning Fish as Sources of Steroid Hormones in the Aquatic Environment." *Environ. Sci. Technol.* **38**(23), 6377-6384.

Kolodziej, E.P., and Sedlak D.L. "Biotransformations of Common Progestin Metabolites Result in Increased Pheromonal Characteristics." *Environ. Sci. Technol.* In preparation. Fall 2004.

## PRESENTATIONS

"Occurrence and Fate of Steroid Hormones with Pheromonal Properties in Northern California." Poster presentation at the Gordon Research Conference, Environmental Sciences: Water, June 27-July 2, 2004.

"Occurrence and Fate of Steroid Hormones in Northern California." Invited oral presentation at the Northern California Chapter of the Society of Environmental Toxicology and Chemistry 14<sup>th</sup> annual meeting, Davis, CA, May 12, 2004.

- 1<sup>st</sup> Place award for Best Student Presentation.

"Moving Beyond Estrogen: Occurrence and Fate of Androgens, Estrogens, and Progestins from Anthropogenic Sources." Invited oral presentation at the American Institute of Chemical Engineers 2003 annual meeting, San Francisco, CA, November 16-21, 2003.

"Occurrence and Fate of Steroid Hormones with Pheromonal Properties from Anthropogenic Sources." Oral presentation at the Society of Environmental Toxicology and Chemistry 24<sup>th</sup> annual meeting, Austin, TX, November 9-13, 2003.

"Fate of Estrogenic Hormones in Effluent-Dominated Surface Waters." Poster session at the Society of Environmental Toxicology and Chemistry 22<sup>nd</sup> annual meeting, Baltimore, MD, November 4-8, 2001.

"Do Synthetic Progestins Interfere with Chemoreception in Spawning Fish?" Oral presentation at the WC<sup>3</sup> Annual Conference, Bodega Bay, CA, March 10-11, 2001.

## REFERENCES

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#### Education

B.S., St. Mary's College, Moraga, California, 1965

M.S., University of Pacific, Dillon Beach, California, 1969

Ph.D., University of Southern California, Los Angeles, California, 1971

#### Awards and Fellowships:

California State Scholarship, 1961

St. Mary's College Scholarship Award, 1961

St. Mary's College Assistantship, 1964

University of Pacific Research Assistantship, 1965

University of Southern California Assistantship, 1967

National Institute of Health Graduate Research Award, 1968

National Science Foundation, Summer Fellowship, 1969

Arthur Vining Davis Fellowship, 1969

#### Positions Held:

Instructor, University of California , Los Angeles, 1968

Senior Research Officer, Marine Studies Group, Ministry for Conservation, Melbourne, Australia,  
1970-1973

Marine Scientist, Lawrence Livermore National Laboratory,  
Livermore, California, 1973-1991

President, Applied Marine Sciences, 1990-

Chief Scientist, *Exxon Valdez* Oil Spill Trustee Council, 1990-2001

Board of Directors of the Romberg Tiburon Center for Environmental Studies, 1993-2002

Board of Directors, Alaska SeaLife Center, 1994-

#### Societies:

American Association for the Advancement of Science

American Chemical Society

American Society of Limnology and Oceanography

Society of Environmental Toxicology and Chemistry

#### Honors:

American Men and Women of Science, 1977

Who's Who in California, 1982

Commendation Letter from US Attorney General, 1992

#### Major Research Interests:

The fate and effects of contaminants(especially petroleum) in the aquatic environment, with emphasis on fish and benthic invertebrates. Radiouclides in aquatic organisms and their effects; the relationships between the activities of xenobiotic-transforming enzymes, contaminant exposure and reproduction in estuarine and coastal fishes; alteration of hormone production and balance by

receptor-mediated contaminant effects; the effects of oil spills on ecosystems; the detection and quantification of polynuclear aromatic hydrocarbons and chlorinated aromatic hydrocarbons in sediments and organisms; the roles of organic enrichment and toxicity in the development of marine benthic communities in contaminated sediments; the degradation and utilization of petroleum hydrocarbons in sediments; the utilization of petroleum and sewage carbon in nearshore marine food webs; The use of natural isotopes in food webs as tracers; benthic communities and processes in natural petroleum seeps; benthic-pelagic coupling; biogeochemistry of oil-contaminated sediments; chemical tracers of street runoff; detecting community change in deep-water, hard-bottom communities; effects of contaminated sediments on marine organisms; sediment bioassays; design of programs to detect long-term change in benthic communities; applications of accelerator mass spectrometry in marine ecology.

Other Professional Activities:

Editor, *Marine Environmental Research* , 1987-1993; 2001-  
Editorial Board, *Marine Environmental Research* , 1993-  
Editorial Board, *Aquatic Toxicology*, 1996-  
US Regional Editor, *Marine Environmental Research* , 1980-1986  
Member, Consulting Board, Southern California Coastal Water  
Research Project, 1986-1989  
Member, Environmental Biology Review Panel, US Environmental  
Protection Agency, 1982-1984  
Member, Regional Effects Technical Advisory Committee, California  
Regional Water Quality Control Board, 1983-1986  
Contributor, National Research Council's Report, *Oil In the Sea* , 1985  
Chairman, Technical Advisory Committee, San Francisco Bay-Delta Project, 1987-1989.  
Member, National Research Council, Panel on Particulate Wastes, Committee on Systems  
Assessment of Marine Environmental Monitoring, 1987-1989  
Member, Scientific Peer Review Committee, Orange County Sanitation Districts, 1988-  
Member, Technical Review Committee, San Francisco Bay-Delta Aquatic Habitat Institute,  
1990-  
Member, Striped Bass Health Monitoring Review Committee, California Department of Fish and  
Game and State Water Resource Control Board, 1989-1990  
Member, Steering Committee, University of California at Santa Barbara, MMS Educational  
Initiative, 1990-  
Member, Workshop Panels on Polynuclear Aromatic Hydrocarbons (1988) and Genetic Effects  
of Sediment Contaminants (1990), Corps of Engineers, Waterways Experiment Station  
Participant, Workshop to Assess NOAA's National Status and Trends, Program, 1987  
Invited Speaker, NIEHS Conference on Marine Contaminants, 1986, Research Triangle, North  
Carolina  
Participant, National Research Council, Workshop on Science and Litigation, September,  
1991  
Participant, National Research Council, Workshop on Coastal Science and Policy  
Interactions in the United States, Irvine, CA, 1992  
Presenter, National Research Council, Committee on Exploitation of the Outer  
Continental Shelf, Anchorage, Alaska, 1992  
Co-chair of working group on monitoring options, Workshop on Environmental Monitoring of  
Invited by the Minister of Transport in Great Britain and Governor of Alaska to visit the site of the  
*Sea Empress* Oil Spill in Wales, March, 1996  
Member, Scientific Advisory Committee, UC System-wide Lead Campus Program in  
Exotoxicology, UC Davis. 1998-  
Member, Public Advisory Committee, University of California Toxic Substances Research and  
Teaching Program, 2000-  
Member, Interim Science Board, CALFED Restoration Program, Sacramento, CA. 1999-  
Member, National Research Council , Committee on Oil in the Sea: Inputs, Fates, and Effects,  
2000  
Review of proposals, papers and dissertations for:

Environmental Protection Agency, Environmental Science Grants Program  
 Environmental Protection Agency, National Center for Environmental Research and Quality  
 Assurance  
 National Oceanographic and Atmospheric Administration  
 National Science Foundation  
 National Research Council  
 Natural Environment Research Council (United Kingdom)  
 European Congress of Limnology and Oceanography  
 International Joint Commission (Great Lakes)  
 Aquatic Habitat Institute  
 Citizens for a Better Environment  
 Massachusetts Sea Grant  
 Georgia Sea Grant  
 State of Alaska  
 Estuarine Research Federation  
 Department of Energy  
 Marine Review Committee, Inc.  
 National Undersea Research Center  
 University of California, Davis  
 University of California, Santa Barbara  
 University of Maryland  
 CRC Press  
 American Chemical Society, Petroleum Research Fund  
 Southern California Coastal Water Research Project  
 Hudson River Foundation  
 John Simon Guggenheim Foundation  
 San Francisco Estuary Project, Gaps in Knowledge Program  
*Aquatic Toxicology*  
*Canadian Journal of Fisheries and Aquatic Sciences*  
*Environmental Toxicology and Chemistry*  
*Hydrobiologia*  
*Journal du Conseil* (international Council for the Exploration of the Sea)  
*Journal of Experimental Marine Biology and Ecology*  
*Marine Biology*  
*Marine Ecology Progress Series*  
*Marine Environmental Research*  
*Marine Pollution Bulletin*  
*Marine Environmental Research*  
*Science*

#### SELECTED PUBLICATIONS

1975 R.B. Spies Structure and function of the head of flabelligerid polychaetes.  
*J. Morph.* 147, 187-208.

1975 R.B. Spies Uptake of technetium from seawater by red abalone *Haliotis rufescens*,  
*Health Phys.* 29, 695-699.

1976 F. Milanovitch, R.B. Spies, M.S. Giam and E.E. Sykes. Uptake of copper by the  
 polychaete *Cirriiformia spirabanchia* in the presence of dissolved organic  
 matter of natural origin. *Estuar. Coast. Mar. Sci.* 4, 585-588.

1979 R.B. Spies and P.H. Davis. The infaunal benthos of a natural oil seep in the  
 Santa Barbara Channel. *Mar. Biol.* 50, 227-237.

- 1980 R.B. Spies, P.H. Davis and D. Stuermer. Ecology of a petroleum seep off the California coast. in *Marine Environmental Pollution* (R. Geyer, Ed.). Elsevier, Amsterdam, pp. 229-263.
- 1980 P.H. Davis and R.B. Spies. Infaunal benthos of a natural petroleum seep: a study of community structure. *Mar. Biol.* 59, 31-41.
- 1981 R. B. Spies, K. Marsh and J. R. Kercher. Dynamics of radionuclide exchange in the calcareous algae *Halimeda* at Enewetak Atoll. *Limnol. Oceanogr.* 26, 74-85.
- 1981 D.H. Steurmer, R.B. Spies and P.H. Davis. Toxicity of Santa Barbara seep oil to starfish embryos. I. Hydrocarbon composition of test solutions and field samples. *Mar. Environ. Res.* 5, 275-286.
- 1982 R.B. Spies and P.H. Davis. Toxicity of Santa Barbara seep oil to starfish embryos. III. Influence of parental exposure and the effects of other crude oils. *Mar. Environ. Res.* 6, 3-11.
- 1982 R.B. Spies, J.S. Felton and L.J. Dillard. Hepatic mixed-function oxidases in California flatfish are increased in contaminated environments and by oil and PCB ingestion. *Mar. Biol.* 70, 117-127.
- 1982 D.H. Steurmer, R.B. Spies, P.H. Davis, D.J. Ng, C.J. Morris and S. Neal. The hydrocarbon chemistry of the Isla Vista Marine Seep Environment. *Mar. Chem.* 11, 413-426.
- 1983 R.B. Spies and D.J. DesMarais. Natural isotope study of trophic enrichment of marine benthic communities by petroleum seepage. *Mar. Biol.* 73, 67-71.
- 1984 R.B. Spies. Benthic-pelagic coupling in sewage affected ecosystems. *Mar. Environm. Res.* 13, 195-230.
1985. P.A. Montagna and R.B. Spies. Meiofauna and chlchlorophyll associated with *Beggiatoa* mats of a natural submarine petroleum seeps. *Mar. Environ. Res.* 16, 231-242.
- 1986 P.A. Montagna, J.E. Bauer, M.C. Prieto, D.H. Hardin and R. B. Spies. Benthic metabolism in a natural coastal petroleum seep. *Mar. Ecol. Prog. Ser.*, 34, 31-40.
- 1987 R. B. Spies. The biological effects of petroleum hydrocarbons in the sea: Assessments from field and microcosms, pp. 411-467 in *Long-term environmental effects of offshore oil and gas development*. D.F. Boesch and N.N. Rabalais, Eds. Elsevier-Applied Sciences, London.
- 1987 R. B. Spies , B. Andresen and D.W. Rice, Jr. Benzthiazoles in estuarine sediments as indicators of street runoff. *Nature* 327: 697-699.
- 1987 P.A. Montagna, J.E. Bauer, J. Toal, D.H. Hardin and R.B. Spies. Temporal variability and the relationship between benthic meiofaunal and microbial populations in a natural coastal petroleum seep. *J. Mar. Res.* 45, 761-789.
- 1987 Melzian, B. D., Zoffman, C. and R.B. Spies. Chlorinated hydrocarbons in lower continental shelf fish collected near the Farallon Islands, California. *Marine Pollution Bull.* 18, 388-393.
- 1988 R. B. Spies, D.W. Rice, Jr. and J.W. Felton. The effects of organic contaminants on reproduction of starry flounder, *Platichthys stellatus* (Pallas) in San Francisco Bay. Part I. Hepatic contamination and mixed-function oxidase(MFO) activity during the reproductive season. *Marine Biology* 98, 181-189.

- 1988 R.B. Spies and D.W. Rice, Jr. The effects of organic contaminants on reproduction of starry flounder, *Platichthys stellatus* (Pallas) in San Francisco Bay. Part II. Reproductive success of fish captured in San Francisco Bay and spawned in the laboratory. *Marine Biology* 98, 191-202.
- 1988 R.B. Spies, D. Hardin and J. Toal. Organic enrichment or toxicity? A comparison of the effects of kelp and crude oil in sediments on the colonization and growth of fauna. *J. Exp. Mar. Biol. Ecol.* 124, 261-282.
- 1988 J.E. Bauer, P.A. Montagna, R.B. Spies, D.H. Hardin and M. Prieto. Microbial biogeochemistry and heterotrophy in sediments of a marine hydrocarbon seep. *Limnol. Oceanogr.* 33, 1493-1513.
- 1988 D.J. H. Phillips and R.B. Spies. Chlorinated hydrocarbons in the San Francisco estuarine ecosystem. *Mar. Poll. Bull.* 19, 445-453.
- 1989 R.B. Spies, D.D. Hardin and J.P. Toal. Organic enrichment or toxicity? A comparison of the effects of kelp and crude oil in sediments on the colonization and growth of benthic infauna. *J. Exp. Mar. Biol. Ecol.* 124, 261-282.
- 1989 R.B. Spies, J.E. Bauer and D. H. Hardin. A stable isotope study of sedimentary carbon utilization by *Capitella* spp.: effects of two carbon sources and geochemical conditions during their diagenesis *Marine Biology* 101: 68-74.
- 1989 R. B. Spies, H. Kruger, R. Ireland and D.W. Rice, Jr. Stable isotope ratios and contaminant concentrations in a sewage-distorted food web. *Mar. Ecol. Prog. Ser.* 54, 157-170.
- 1989 R.B. Spies, D.W. Rice Jr., P.J. Thomas, J.J. Stegeman, J.N. Cross and J.E. Hose. A field test for correlates of poor reproductive success and genetic damage in contaminated populations of starry flounder, *Platichthys stellatus*. *Mar. Environ. Res.* 28: 542-543.
- 1990 R.B. Spies, J.J. Stegeman, D.W. Rice, Jr., B. Woodin, P. Thomas, J.E. Hose, J. Cross and M. Prieto. Sublethal responses of *Platichthys stellatus* to organic contamination in San Francisco Bay with emphasis on reproduction, pp. 87-122, in *Biological Markers of Environmental Contamination*. Lewis Publishers, Chelsea, Michigan .
- 1990 J.E. Bauer, R.B. Spies, J. S. Vogel, D.E. Nelson and J.R. Southon. Radiochemical evidence of fossil hydrocarbon cycling in sediments of a nearshore hydrocarbon seep. *Nature* 348, 230-232.
- 1992 M.J. Melancon, R. Alscher, W. Benson, G. Kruzynski, R.F. Lee, H.C. Sikka, R.B. Spies. Metabolic products as biomarkers, In: *Biomarkers: Biochemical, physiological and histological markers of anthropogenic stress* (Huggett et al., Eds) Lewis Publishers, Boca Raton, Florida.
1993. J.W. Anderson, D.J. Reish, R.B. Spies, M.E. Brady and E.W. Segelhorst. Human impacts, pp. 682-766, In: *Ecology of the Southern California Bight* (, M.D. Daily, J.W. Anderson, and D.J. Reish, eds.) University of California Press, Berkeley, 926 pp.
- 1993 R.B. Spies. So why can't science tell us more about the effects of the Exxon Valdez oil spill ?, pp. 1-5, In: *Exxon Valdez oil spill symposium*, EVOS Trustee Council, Anchorage Alaska.
- 1994 D.W. Rice, C.B. Seltnerich, M.L. Keller, R.B. Spies, and J.S. Felton. Mixed-function oxidase-specific activity in wild and acaged speckled sandabs *Citharichthys stigmaeus* in Elkhorn Slough. *Environ. Poll.* 84, 179-188.
1995. R. Spies. Restoring Prince William Sound. *Science* 269, 1328-1329. (letter)

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

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

1996.R. B. Spies, S. D. Rice, D. A. Wolfe and B. A. Wright. The effects of the *Exxon Valdez* Oil spill on the Alaskan Coastal environment, pp. 1-16, in: Rice, S.D., R.B. Spies, D.A. Wolfe, and B.A. Wright (Eds.) *Exxon Valdez Oil Spill Proceedings, Anchorage, Alaska, 2-5 February 1993*. American Fisheries Society Symposium No. 18.

1997. A.J. Gunther, R.B. Spies, J.J. Stegeman, B. Woodin, D. Carney, J. Oakden, and L. Hain. 1997. EROD activity as an independent measure of contaminant-induced mortality of invertebrates in sediment bioassays. *Marine Environmental Research* 44:41-49.

1997 R.B. Spies and P. Thomas. Reproductive and endocrine status of mature female kelp bass *Paralabrax clathratus* from a contaminated site in the Southern California Bight and estrogen receptor binding of DDTs, Chapter 9, in *Chemically-induced alterations in functional development and reproduction of fishes*, R.M. Rolland, M. Gilbertson and R.E. Peterson (Eds.) Society of Environmental Contamination and Toxicology, Technical Publication Series, SETAC Press, Pensacola, FL.

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## ***Education***

- 1994-2000 | **Michigan State University, East Lansing, Michigan**  
Doctorate of Philosophy  
Environmental Toxicology and Zoology  
Dissertation Title: ***Instrumental and Bioanalytical Measures of Endocrine Disruptors in Water***  
*Advisor:* Dr. John P. Giesy (Distinguished Professor)
- 1990-1994 | **Thiel College, Greenville, Pennsylvania**  
Department of Chemistry  
Bachelor of Arts: Magna Cum Laude  
Major: Chemistry  
Minor: Biology

## ***Grants Funded***

- 2004-2006 | **Principal Investigator – American Water Works Association Research Foundation and WateReuse Foundation:** \$737,000 “Toxicological Relevance of EDC and Pharmaceuticals in Drinking Water” Project #3085
- 2004-2006 | **Co-Principal Investigator – WateReuse Foundation:** *Colorado School of Mines as PI* “Development of Indicators and Surrogates for Chemical Contaminant Removal during Wastewater Treatment and Reclamation”
- 2004-2006 | **Co-Principal Investigator – WateReuse Foundation:** *Carollo Engineers as PI* “Reclaimed Water Aquifer Storage and Recovery: Potential Changes in Water Quality”
- 2004-2006 | **Co-Principal Investigator – Water Environment Research Foundation:** \$202,000 *Colorado School of Mines as PI.* “Contributions of Household Chemicals to Sewage and their Relevance to Municipal Wastewater Systems and the Environment”
- 2002-2004 | **Principal Investigator - American Water Works Association Research Foundation:** \$350,000 “Evaluation of Conventional and Advanced Treatment Processes to Removal Endocrine Disruptors and Pharmaceutically Active Compounds” Project #2758
- 2001-2004 | **Principal Investigator - Strategic Environmental Research and Development Program (for Department of Defense):** \$300,000 “Toxicological Impact of Ammonium Perchlorate on Fish (project id#1222)”
- 1998-2000 | **Principal Investigator - Southern Nevada Water Authority/U.S. Bureau of Reclamation/National Park Service:** \$255,000 “Toxicity Identification and Evaluation of Xenobiotic Compounds in Lake Mead, Nevada”
- 1998-2000 | **Principal Author - Chemical Manufacturers Association:** \$206,000 “Identification and Quantitation of Alkylphenols from Fish Tissues”
- 1997 | **Principal Author - Las Vegas Valley Water District:** \$14,600 “Screening of Drinking Water for Possible Endocrine Disrupting Compounds”
- 1997 | **Principal Author - Chemical Manufacturers Association:** \$11,250 Instrument grant for alkylphenol analyses

## ***Recent Volunteer Efforts***

- 2004-2006 | **Federal Advisory Committee Member:** Endocrine Disruptor Methods Validation Advisory Committee (EDMVAC).

2004-Present	<b>American Water Works Association:</b> Source Water Protection Committee – Vice-Chair.
2002-Present	<b>American Water Works Association:</b> Organic Contaminants Research Committee – Vice-Chair.
2002-Present	<b>Henderson Blue Ribbon Commission:</b> Member of special committee to promote educational excellence in Southern Nevada.
2001-2004	<b>National Advisory Council for Environmental Policy and Technology:</b> Member of the US EPA Federal Advisory Committee “ <i>Endocrine Disruptor Methods Validation Subcommittee (EDMVS)</i> ”.
2001-2004	<b>American Water Works Association Research Foundation:</b> Project Advisory Committee member “Assessment of Source Waters and Drinking Waters for Endocrine Disruptors”.
2001-Present	<b>Water Environment Research Foundation:</b> Project Subcommittee member “The Use of Bioassays and Chemical Measurements to Assess the Removal of Endocrine Disrupting Compounds in Water Reclamation Systems, 01-HHE-20T”.
2000-2001	<b>American Water Works Association:</b> Endocrine Disruptor & the Water Industry Symposium planning committee.

**Research Experience**

2000-Present	<p><b>Research and Development – Project Manager.</b> Southern Nevada Water Authority, Las Vegas, Nevada.</p> <ul style="list-style-type: none"> <li>• <i>Research Efforts:</i> Fate and transport of trace concentrations of xenobiotics in water. Use of both LC and GC MS for identification and quantitation of water contaminants. Particular emphasis on endocrine disrupting compounds, pharmaceuticals and personal care products. Investigations of emerging drinking water concerns and development of corrective action plans. Design and implementation of pilot and bench scale water treatment systems. Generating research proposals for competitive grants.</li> </ul>
1994-2000	<p><b>Graduate Student.</b> Michigan State University, East Lansing, Michigan. Department of Zoology and Institute of Environmental Toxicology</p> <ul style="list-style-type: none"> <li>• <i>Principal Investigator:</i> Dr. John P. Giesy</li> <li>• <i>Responsibilities:</i> Trace analysis of environmental samples: PCBs, pesticides, PCDD/F, non-ionic surfactants and metabolites; QA/QC; method development for HPLC, GC/MS, GC/ECD, GC/FID, and GPC techniques. Use of cellular bioassays to detect endocrine disrupting chemicals.</li> <li>• <i>Research Efforts:</i> Novel solid-phase extraction and fractionation techniques; molecular modeling; toxicity identification and evaluation (TIE) assays.</li> </ul>
1995 – 1998	<p><b>Environmental Consultant.</b> Giesy Ecotoxicology, Inc., Williamston, Michigan</p> <ul style="list-style-type: none"> <li>• <i>Responsibilities:</i> Literature searching, compiling, and reviewing; formulation of ecological risk assessments; computer modeling.</li> </ul>
Summer 1995	<p><b>Research Internship.</b> Bayer Corp., Biotechnology Division, Leverkusen, Germany</p> <ul style="list-style-type: none"> <li>• <i>Project:</i> R&amp;D of anti-viral pharmaceuticals.</li> <li>• <i>Responsibilities:</i> Synthesis of RNA, DNA, and PNA oligonucleotides structurally modified for improved cellular uptake; product purification and structural confirmation using HPLC and MALDI-MS; DNA and RNA sequencing.</li> </ul>
Summer 1994	<p><b>Research Internship.</b> Bayer Corp., New Martinsville, West Virginia</p> <ul style="list-style-type: none"> <li>• <i>Project:</i> R&amp;D of open path Fourier Transform infrared spectrophotometric remote gas analyzer.</li> <li>• <i>Responsibilities:</i> Analyzer diagnostics; process development; establishment of spectra library.</li> </ul>

**Additional Relevant Experience**

2000 – Present	<b>Adjunct Faculty.</b> University of Nevada, Las Vegas. Act as a committee member for various graduate research programs. Aid in research efforts of faculty and students. Instruct classes and serve as a visiting lecturer.
2000 – Present	<b>Adjunct Faculty.</b> Community College of Southern Nevada, Las Vegas, Nevada.

- Instruct undergraduate science classes. Work with other faculty on local environmental issues.
- 1998 – Present **Owner/Consultant.** Total Environmental Solutions Inc., Henderson, Nevada. Providing consulting services on environmental and analytical issues.
- 1997 – Present **Peer Reviewer.** Acted as a peer reviewer for the following journals: *Analytical Chemistry, Environmental Science and Technology, Journal of Agricultural and Food Chemistry, Journal of the American Water Works Association, and the International Journal of Remote Sensing.*
- 2000 **Invited Speaker.** National Public Radio (NPR) recorded in Las Vegas, Nevada. Interview regarding pharmaceuticals and personal care products in Lake Mead, Nevada.
- 1997 **Reviewer.** Partners in Research, Arlington, Virginia. Assisted in peer review of EPA water criteria documents.

### **Publications**

- 2004 Snyder SA, Vanderford BJ, Rexing DJ. *Trace Analysis of Bromate, Chlorate, Iodate, and Perchlorate in Natural and Bottled Waters.* Environmental Science and Technology (in review)
- 2004 Snyder SA, Leising J, Westerhoff P, Yoon Y, Mash H, Vanderford BJ. *Biological Attenuation of EDCs and PPCPs: Implications for Water Reuse.* Ground Water Monitoring and Remediation 24 (2)
- 2004 Yoon Y, Westerhoff P, Yoon J, Snyder SA. *Removal of 17- $\beta$  Estradiol and Fluoranthene by Nanofiltration and Ultrafiltration.* Journal of Environmental Engineering (in press)
- 2003 Snyder SA, Vanderford BJ, Pearson RA, Quinones O, Yoon Y. *Analytical Methods to Measure Endocrine Disrupting Compounds in Water.* Practice Periodical of Hazardous, Toxic, and Radioactive Waste Management 7 (4):224-234
- 2003 Snyder SA, Westerhoff P, Yoon Y, Sedlak DL. *Pharmaceuticals, Personal Care Products, and Endocrine Disruptors in Water.* Environmental Engineering Science 20 (5):449-469
- 2003 Vanderford BJ, Pearson RA, Rexing DJ, Snyder SA. *Analysis of Endocrine Disruptors, Pharmaceuticals, and Personal Care Products in Water using Liquid Chromatography/Tandem Mass Spectrometry.* Analytical Chemistry 75 (22):6265-6274
- 2003 Vanderford BJ, Pearson RA, Cody RB, Rexing DJ, Snyder SA. *Determination of an Unknown System Contaminant using LC/MS/MS.* In Liquid chromatography/mass spectrometry, MS/MS and Time-of-Flight MS, Ferrer, I. and Thurman, E.M. (eds.), Symposium Series 850; American Chemical Society, Washington, DC, Chapter 6:96-108
- 2003 Yoon Y, Westerhoff P, Snyder SA, Esparza M. *HPLC-Fluorescence Detection and Adsorption of Bisphenol A, 17 $\beta$ -Estradiol, and 17 $\alpha$ -Ethinyl Estradiol on Powdered Activated Carbon.* Water Research 37(14):3530-3537
- 2002 Snyder SA, Vanderford BJ, Pearson R, Rexing DJ. *Analytical Methods for Measuring Endocrine Disruptors in Water.* Water Quality Technology Conference Proceedings 2002.
- 2001 Snyder SA, Keith TL, Snyder EM, Giesy JP. *Bioconcentration of Nonylphenol in Fathead Minnows (*Pimephales promelas*).* Chemosphere 44(8):1697-1702.
- 2001 Snyder SA, Villeneuve DL, Snyder EM, Giesy JP. *Identification and Quantification of Estrogen Receptor Agonists in Wastewater Effluents.* Environmental Science and Technology 35(18):3620-3625.
- 2001 Snyder SA, Keith TL, Naylor CG, Staples CA, Giesy JP. *Identification and Quantification Method for Nonylphenol and Lower Oligomer Nonylphenol Ethoxylates in Fish Tissues.* Environmental Toxicology and Chemistry 20(9):1870-1873.
- 2001 Snyder SA, Kelly KL, Grange AH, Sovocool GW, Snyder EM, Giesy JP. *“Pharmaceuticals and Personal Care Products in the Environment: Methods,*

- Analyses, and Sources.”* In Pharmaceuticals and Personal Care Products in the Environment: Scientific and Regulatory Issues, Daughton, C.G. and Jones-Lepp, T. (eds.), *Symposium Series 791*; American Chemical Society, Washington, DC, Chapter 7:116-139.
- 2001 Nichols KM, Snyder EM, Snyder SA, Miles-Richardson SR, Pierens S, Giesy JP. *Effects of Nonylphenol Ethoxylate (NPEO) Exposure on Reproductive Output and Bioindicators of Environmental Estrogen Exposure in Fathead Minnows, Pimephales promelas*. *Environmental Toxicology and Chemistry* 20(3):510-522.
- 2001 Keith TL, Snyder SA, Naylor CG, Staples CA, Summer CL, Kannan K, Giesy JP. *Identification and Quantitation of Nonylphenol Ethoxylates and Nonylphenol in Fish Tissues from Michigan*. *Environmental Science and Technology* 35(2):10-13.
- 2000 Snyder SA, Snyder EM, Villeneuve DL, Kannan K, Villalobos SA, Blankenship A, Giesy JP. “*Instrumental and Bioanalytical Measures of Endocrine Disruptors in Water.*” In Analysis of Environmental Endocrine Disruptors, Keith, L.H., Jones-Lepp, T.L., and Needham, L.L. (eds.), *Symposium Series 747*, American Chemical Society, Washington, DC, Chapter 6:73-95.
- 2000 Roefer PA, Snyder SA, Zegers RE, Rexing DJ, Fronk JL. *Endocrine-Disrupting Chemicals in a Source Water*. *Journal of the American Water Works Association* 92(8):52-58.
- 2000 Giesy JP, Pierens SL, Snyder EM, Miles-Richardson S, Kramer VJ, Snyder SA, Nichols KM, Villeneuve DL. *Effects of 4-Nonylphenol on Fecundity and Biomarkers of Estrogenicity in Fathead Minnows (Pimephales promelas)*. *Environmental Toxicology and Chemistry* 19(5):1368-1377.
- 2000 Snyder EM, Snyder SA, Giesy JP, Blonde SA, Hurlburt GK, Summer CL, Mitchell RR, Bush DM. *SCRAM: A Scoring and Ranking System for Persistent, Bioaccumulative and Toxic Substances for the North American Great Lakes. Part I. Structure of the Scoring and Ranking System*. *Environmental Science & Pollution Research* 7(1):51-61.
- 2000 Snyder EM, Snyder SA, Giesy JP, Blonde SA, Hurlburt GK, Summer CL, Mitchell RR, Bush DM. *SCRAM: A Scoring and Ranking System for Persistent, Bioaccumulative and Toxic Substances for the North American Great Lakes. Part II. Bioaccumulation Potential and Persistence*. *Environmental Science & Pollution Research* 7(2):116-221.
- 2000 Snyder EM, Snyder SA, Giesy JP, Blonde SA, Hurlburt GK, Summer CL, Mitchell RR, Bush DM. *SCRAM: A Scoring and Ranking Model for Persistent, Bioaccumulative and Toxic Substances for the North American Great Lakes. Part III. Acute and Subchronic or Chronic Toxicity*. *Environmental Science & Pollution Research* 7(3):176-184.
- 2000 Snyder EM, Snyder SA, Giesy JP, Blonde SA, Hurlburt GK, Summer CL, Mitchell RR, Bush DM. *SCRAM: A Scoring and Ranking Model for Persistent, Bioaccumulative and Toxic Substances for the North American Great Lakes. Part IV. Results from Representative Chemicals, Sensitivity Analysis, and Discriminatory Power*. *Environmental Science & Pollution Research* 7(4):219-224.
- 1999 Snyder SA, Keith TL, Verbrugge DA, Snyder EM, Gross TS, Kannan K, Giesy JP. *Analytical Methods for Detection of Selected Estrogenic Compounds in Aqueous Mixtures*. *Environmental Science and Technology* 33(16):2814-2820.
- 1999 Snyder SA and Snyder EM. *Bad Medicine*. *Resource* 6(5):7-8.
- 1999 Khim JS, Villeneuve DL, Kannan K, Lee KT, Snyder SA, Koh CH, Giesy JP. *Akylphenols, Polycyclic Aromatic Hydrocarbons (PAHs), and Organochlorines in Sediment from Lake Shihwa, Korea: Instrumental and Bioanalytical Characterization*. *Environmental Toxicology and Chemistry* 18:2424-2432.
- 1999 Miles-Richardson SR, Pierens SL, Nichols KM, Kramer VJ, Snyder EM, Snyder SA, Render JA, Fitzgerald SD, Giesy JP. *Effects of Waterborne Exposure to 4-Nonylphenol and Nonylphenol Ethoxylate on Secondary Sex Characteristics and Gonads of Fathead Minnows (Pimephales promelas)*. *Environmental Research A*

- 80:S122-S137.  
1997 Froese KL, Verbrugge DA, Snyder SA, Tilton F, Tuchman M, Ostaszewski A, Giesy JP. *PCBs in the Detroit River Water Column*. *Journal of Great Lakes Research* 23(4):440-449.

### ***Professional Affiliations***

- 2000 – Present American Water Works Association  
1997 – Present American Association for the Advancement of Science  
1996 – Present Sigma Xi  
1995 – Present Society of Environmental Toxicology and Chemistry  
1994 – Present Phi Lambda Upsilon, Honorary Chemical Society  
1993 – Present Alpha Chi, National College Honor Scholarship Society  
1991 – Present American Chemical Society

### ***Academic Awards***

- 1998 CGL-SETAC Student Platform Presentation Award  
1998 BASF Student Travel Award  
1998 ACS Committee on Science –ACS National Meeting Award  
1997 SETAC Travel Award  
1994 ACS Polymer Division Outstanding Performance in Organic Chemistry Award  
1994 Society for Analytical Chemists of Pittsburgh College Chemistry Award  
1994 Dean's Key Award (8 semesters of Dean's List recognition)  
1991-1994 Thiel College Honors Society  
1993 ACS Undergraduate Award in Analytical Chemistry  
1992-1993 Thiel Science Caucus Scholarship

### ***References***

#### **Dr. John P. Giesy, Distinguished Professor**

Aquatic Toxicology Laboratory  
Department of Zoology  
218C National Food Safety and Toxicology Center  
Michigan State University  
East Lansing, Michigan 48824

#### **Dr. Kevin L. Kelly, Research Chemist**

Bureau of Reclamation  
Denver Federal Center  
Bldg. 56 Room 2300  
Denver, Colorado 80225

#### **David J. Rexing, Water Quality Research and Development Manager**

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## Identifying the Causes of Feminization of Chinook Salmon in the Sacramento and San Joaquin River System: signature

This proposal is for the Science Program 2004 solicitation as prepared by Sedlak, David L.



The applicant for this proposal must submit this form by printing it, signing below, and faxing it to +1 877-408-9310.

*Failure to sign and submit this form will result in the application not being considered for funding.*

The individual signing below declares that:

- all representations in this proposal are truthful;
- the individual signing the form is authorized to submit the application on behalf of the applicant (if applicant is an entity or organization);
- the applicant has read and understood the conflict of interest and confidentiality discussion under the Confidentiality and Conflict of Interest Section in the main body of the PSP and waives any and all rights to privacy and confidentiality<sup>1</sup> of the proposal on behalf of the applicant, to the extent provided in this PSP; and
- the applicant has read and understood all attachments of this PSP.

**proposal title:** Identifying the Causes of Feminization of Chinook Salmon in the Sacramento and San Joaquin River System

**proposal number:** 2004.01-0111

**submitter:** Sedlak, David L (sedlak@ce.berkeley.edu)

**Jannet Kim  
Senior Research Administrator**

11/5/05

applicant signature

date

Regents of the University of California

University of California, Berkeley

printed name of applicant

applicant organization