

## Evaluating bioavailability of pyrethroids in aquatic systems

### A. Experimental Overview:

1. Phase 1. Simultaneous measurements of dissolved concentration and toxicity in model suspended sediment solution test systems.
  - i. Objective: Evaluate bioavailability of pyrethroids in aqueous systems, and develop/validate a model that enables prediction of toxicity using LC50 values (determined in water) and basic properties of the aqueous sample (e.g., suspended solid content and DOM).
2. Phase 2. Extend results from Phase 1 to bed sediments. This will include simultaneous measurements of dissolved concentration of pyrethroid insecticides in sediment porewater by using a “biomimic” sampling method and pyrethroid bioaccumulation/acute toxicity to benthic invertebrates by using standard bioassays in simulated sediment test systems.
  - i. Objective: Evaluate bioavailability of pyrethroids in sediment systems, and characterize dependence of bioaccumulation and acute toxicity of pyrethroids in benthic invertebrates on sediment properties.

### B. Hypothesis

1. Only dissolved phase concentration contributes to aquatic toxicity for water-column organisms such as *Ceriodaphnia dubia*;
2. The dissolved-phase fraction ( $f_{dissolved}$ ) is a function of the characteristics and level of suspended solids and dissolved organic matter, which may be estimated from the following relationship, where [SS] is suspended solid content, [DOC] is DOC concentration,  $k_d$  is partition coefficient between the dissolved phase and the suspended solids, and  $k_{DOC}$  is the partition coefficient between the dissolved phase and DOC:

$$f_{dissolved} = \frac{1}{1 + k_d[SS] + k_{DOC}[DOC]}$$

3. Bioavailability is proportional to  $f_{dissolved}$ , and the actual toxicity in systems with suspended solids and DOM may be therefore estimated by dividing the reference LC50 (measured in clear water) over  $f_{dissolved}$ . However, it is realized that this may not be a straight 1:1 relationship. The measured data can be used to validate this model.
4. Only dissolved phase concentration in sediment porewater contributes to aquatic toxicity for benthic invertebrates such as *Chironomus tentans* and *Hyallela azteca*;
5. The dissolved-phase fraction ( $f_b$ ) in sediment porewater is a function of the sediment properties (e.g., organic carbon content and clay content) and level of dissolved organic matter in the porewater, which may be estimated from the following relationship, where  $\rho_s$  is sediment bulk density, [DOC] is dissolved organic carbon concentration in the porewater,  $k_d$  is partition coefficient between the dissolved phase and the bulk sediment, and  $k_{DOC}$  is the partition coefficient between the dissolved phase and DOC in the porewater:

$$f_b = \frac{1}{1 + k_d\rho_s + k_{DOC}[DOC]}$$

6. Bioavailability is equal to or proportional to  $f_b$ . Conventional analysis after extraction of whole sediment will yield total chemical concentration that cannot be used to predict toxicity for benthic organisms. Analysis of porewater after centrifugation may still provide inaccurate estimate as dissolved organic matter in the porewater may render a fraction

of the porewater concentration unavailable. However, the dissolved concentration may be measured by a “biomimic” sampling method such as solid phase microextraction (SPME) that selectively measures the freely dissolved concentration.

### C. Approach

1. Phase 1, Water Column: characterize partitioning of pyrethroids between suspended solids/DOM and the dissolved phase, and its relationship to acute aquatic toxicity in test systems with different suspended solid/DOM types and levels. Compare dissolved concentration at which significant acute toxicity is observed in suspended sediment system to effect concentration (LC50) determined in pure water.
2. Phase 2, Bed Sediment: develop and validate the use of polydimethylsiloxane (PDMS) polymer coated fibers to measure the bioavailable concentration in sediment porewater, and to correlate PDMS fiber measured concentrations with bioaccumulation potential and acute toxicity for benthic invertebrates as *Chironomus tentans* and *Hyallela azteca*. PDMS is selected because it is a non-polar polymer and its properties mimic the lipid tissue of a benthic invertebrate.

### D. Materials

1. Phase 1, Water column. 4 sediments to be taken from both agricultural and urban watersheds, and with a range of OC contents. Properties will be analyzed using standard methods. One set of sediment samples will be “washed” repeatedly with 0.01N  $\text{CaCl}_2$  to remove/minimize DOC effects, and the other set will be used without this treatment, so that the role of DOC may be determined. Esfenvalerate and permethrin will be used in these experiments.
2. Phase 2, Sediment. This project will include tiered experiments with the ultimate goal of developing SPME as a robust biomimic method for detecting free concentrations of pyrethroids in sediment and validating the developed methods for predicting bioavailability of sediment-borne pyrethroids. It will consist of three sets of experiments: method development and optimization experiments, bioaccumulation validation experiments, and acute toxicity validation experiments. The study will consider 2 pyrethroid insecticides (bifenthrin and permethrin) that are commonly used in California, 2-3 sediment types, and 2 benthic organisms (*Chironomus tentans* and *Hyallela azteca*). Proper statistics will be used throughout the experiments to assure statistically supported data interpretation.

### E. Experiments

1. Phase 1, Water column
  - a. *Adsorption coefficient measurement*: Conduct a batch adsorption experiment to measure sorption coefficient  $K_d$  and  $K_{DOC}$  for the chemicals and sediments to be used in the following experiments. Include 5 concentration steps for the isotherm and estimate the partition coefficients through linear regression. Three replicates for each concentration step.  $K_{DOC}$  will be determined from the dissolved concentration in the supernatant (measured by SPME) and DOC of the supernatant.
    - i. 4 (sediments) x 2 pesticides x 5 concentrations x 3 replicates = 120 samples
    - ii. Time required: 2 month, including methodology development (for SPME), analysis (DOC, dissolved concentration, supernatant concentration, and adsorbed concentration).
  - b. *Toxicity and Bioavailability Evaluation, Test protocol and general design*: Standard 96-h acute toxicity assay with *C. dubia* neonates (<24 h old). Use the measured  $K_d$  and  $K_{DOC}$  to establish the dissolved phase concentrations and other conditions to be used in the study. The general approach is to measure LC50 values while varying the type (4 sediments x 2 pre-treatments = 8 types) and level (4 levels, 0, 20, 50, and 125

mg/L) of sediment present (suspended) in water. Simultaneous measurement of total (by solvent extraction) and dissolved (by SPME) concentrations will be made for selected samples at selected time intervals.

- c. *General LC50 procedures*: Each LC50 measurement will include 6 concentration levels corresponding to dissolved concentration = 0.0, 0.2, 0.5, 1.0, 2.0, 3.0 of reference LC50 value (determined in clear water) and 4 replicates at each concentration. The assay will last for 96 h under static or semi-static (solution changed periodically) conditions. The volume of test solution will be 15 ml, and 5 *C. dubia* neonates will be placed in each test container. Therefore, to obtain one LC50 value, 6 (concentrations) x 4 replicates = 24 vials will be needed, and the test will take about 1 wk. Additional vials will be also needed for SPME and solvent extraction analysis. It is expected that two LC50 assays can be run in 1 week, which should leave adequate time for SPME analysis and total concentration measurement. LC50 values will be estimated using a proper software.
- d. *Pesticide spiking consideration*: To minimize heterogeneity between samples, sediment and water (plus salts for organism) will be mixed in a large glass container (e.g., 1 L glass bottle). The pesticide will be added and mixed for 24 h to allow equilibrium to reach between the different phases. The test sample (15 ml each) will be dispensed out from this mixture.
- e. *Estimated workload*: It is likely that some of the assays will have to be repeated as the concentration range often needs be refined to obtain the LC50 curve. The actual assays may be 1.5-2 times of the number of assays indicated below.

Number of LC50 assays = 2 pesticides x 8 sediment types x 4 sediment loadings = 64 assays. However, since the 0 sediment loading treatment only needs to be run once for each pesticide, the total number of assays will be  $48 + 2 = 50$ . Assuming that 2 assays may be run in 1 week, 25 weeks will be needed to complete these assays. Assuming the repetition is needed for some assays, the total number of assays will be around 75, which will take 36 weeks or 9 months.

## 2) Phase 2, Sediment

- a. *Developing SPME-based Biomimic Sampling Methods*: The first step is to develop and test SPME-based methods for detecting free chemical concentrations of sediments containing residues of pyrethroid insecticides. Based on existing literature and preliminary experiments, we intend to use a quartz-core optical fiber coated with 30- $\mu$ m PDMS as the SPME sampling probe. A bulk quantity of this fiber will be ordered from a commercial source (e.g., Polymicro Technologies, LLC, in Phoenix, AZ). The fiber will be cut into 5-cm pieces in the lab, and activated by heating at  $>200$  °C before use. Experiments will be conducted to determine the partition kinetics, reproducibility and linearity of SPME sampling by using disposal fibers. This method will then be applied to sediment samples and compared with conventional methods. If SPME detects only the free pesticide concentration in the interstitial water, the pesticide concentration ( $C_b$ ) measured by PDMS will be smaller than the whole porewater concentration ( $C_b + C_{DOM}$ ) and much smaller than the whole sediment concentration ( $C_s + C_b + C_{DOM}$ ) measured by a whole sediment extraction method.
- b. *Bioaccumulation Validation Experiments* The next step in validating that SPME detects bioavailable concentration is to establish a relationship between SPME-measured concentrations with pesticide uptake by organisms. In these experiments, sediment samples will be treated with  $^{14}$ C-bifenthrin or permethrin at a sublethal concentration, and the test organisms (*C. tentans* and *H. azteca*) will be exposed to the sediment samples. The overall conditions will follow standard effluent toxicity assays. At the end of the incubation, the organisms will be recovered and body residues will be determined by counting  $^{14}$ C activity after the organisms are combusted in a Biological Sample Oxidizer. The pesticide concentration on the PDMS fiber can

be determined by a similar approach, or by activity counting following solvent extraction. Regression analysis may then be performed between the measurements to determine if SPME selectively detects the concentration available for bioaccumulation.

- c. *Acute Toxicity Validation Experiments* A more useful application of biomimic sampling methods will be for *determination* or prediction of common ecotoxicological endpoints such as acute toxicity. In the last set of experiments, we will apply the developed SPME methods in bioassays for measuring acute toxicity of pyrethroid insecticides to the sediment-dwelling invertebrates *C. tentans* and *H. azteca*. Sediment samples will be spiked with bifenthrin or permethrin at a range of concentrations, and the standard protocols for 10-day static acute toxicity test will be followed to obtain LC50 values. Test animals and PDMS fibers will be simultaneously introduced into the sediment layer. At the end of the 10-d incubation, the organisms will be recovered and mortality determined. The PDMS fibers will be retrieved and the accumulated pesticide will be analyzed. Two methods will be used for calculating LC50 ( $\mu\text{g}/\text{kg}$ ) for each compound. In the first method, LC50 will be first calculated using the nominal concentrations (i.e., spiked concentrations, or  $C = C_S + C_{DOM} + C_b$ ). In the second method, LC50 will be calculated using the SPME-measured concentrations, or  $C_b$ . If a higher LC50 is obtained for the nominal concentration than for the SPME-measured concentration, it may be concluded that adsorption to the sediment phase or DOM has reduced the bioavailability or toxicity of the pyrethroid insecticide. In addition, if the SPME-measured concentration is solely responsible for the observed toxicity, then the LC50 value should be independent of the sediment type when  $C_b$  is used for the calculation.