Defining Equilibration Time of Spiked Sediments for Use in Whole Sediment Toxicity Testing: A Post Hoc Assessment

M.J. Bradley Smithers Viscient LLC, Wareham, MA

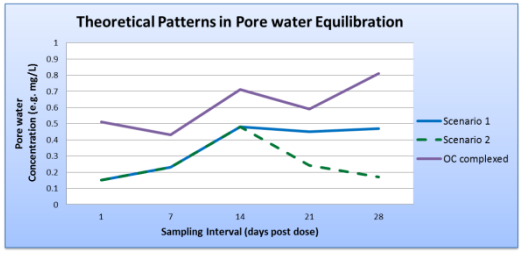
Abstract

Spiked whole sediment toxicity testing in support of US pesticide registration typically utilizes a prolonged aging period of spiked sediments prior to organism exposure, during which concentrations of the applied test compound presumably achieve a state of equilibrium between sediment and pore (interstitial) water. In recent years, regulators have requested that compound-specific equilibration periods be defined prior to organism exposure, as opposed to using default aging periods (e.g., 30-days) described in older test methods. A test design for determining a compound-specific equilibration period can be conceptualized relatively easily, however, interpretation of the results can be difficult due to the adsorptive qualities of the compound, organic carbon content of the sediment-pore water matrices, and the conventional analytical methods employed (e.g., liquid-liquid extraction). While partitioning of a compound with a relatively low adsorption coefficient can be interpreted rather clearly, compounds with a higher adsorption coefficient may result in more variable data, which also may not be considered realistic or relevant to anticipated aqueous concentrations (e.g., measured values exceeding solubility). While many data sets can appear to be random or ambiguous, equilibrium partitioning theory can aid in drawing objective conclusions of time to equilibration depending on compound physico-chemical characteristics and pore water organic carbon (OC) content.

Standard Study Design

- Sediment is spiked at a toxicologically relevant concentration (e.g., 1 to 100 mg/kg dry weight).
- Sediment is mixed and stored refrigerated.
- On days 1, 7, 14, 21, and 28 post-dose, the sediment is mixed and sampled in triplicate.
- Sediment samples are centrifuged at 10,000 *g* for 30 minutes to generate pore water samples which are subsequently analyzed for test compound concentrations

Defining "Equilibration"



- Equilibration is most easily defined by a "steady-state" in pore water concentration (Scenario 1).
- Alternatively, equilibration may be defined by the peak pore water concentration for compounds which may not be as stable over the study period (Scenario 2).
- Chemical analyses which are complexed by the OC content of the pore water may yield no clear equilibration point due to varying OC concentrations over time.

Application of Equilibrium Partitioning Theory

- Freely dissolved concentration of a compound in pore water (C_{FD}) can be estimated based on measured compound concentrations (sediment or total pore water), organic carbon content of the matrix, available adsorption coefficients, and the following equations[1]:

$$C_{FD} = \frac{Conc._{Sediment}}{OC\ Fraction \times K_{OC}} \quad \text{or} \quad C_{FD} = \frac{Conc._{pore\ water}}{DOC \times K_{DOC} + 1}$$

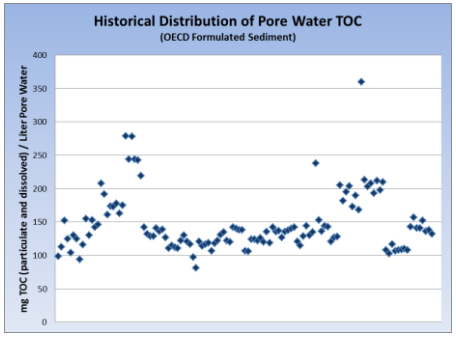
- The above calculations can be combined to calculate an estimated theoretical total pore water concentration (*freely dissolved + OC complexed*) based on sediment concentration, using sediment TOC, pore water TOC (dissolved and particulate), and K_{OC}:

$$Total\ Conc._{pore\ water} = \frac{Conc._{Sediment}}{OC\ Fraction \times K_{OC}} \times (Pore\ water\ TOC \times K_{OC} + 1)$$

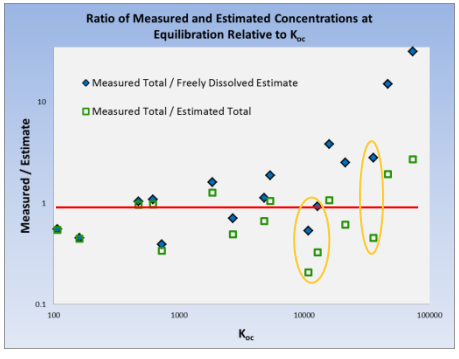
- The calculated total pore water concentration establishes a theoretical "target" for measured pore water concentrations during an assessment of time to equilibration, which can assist an objective interpretation.

[1]U.S. EPA. 2012. Equilibrium Partitioning Sediment Benchmarks (ESBs) for the Protection of Benthic Organisms: Procedures for the Determination of the Freely Dissolved Interstitial Water Concentrations of Nonionic Organics. U.S. Environmental Protection Agency. Office of Research and Development. EPA/600/R-02/012

General Data Review



- Mean DOC is ~ 100 mg/L, however TOC (particulate and dissolved) is more variable with a mean of ~150 mg/L.
- Variability of TOC can be a function of batch characteristics and effectiveness of centrifugation.
- Because TOC will confound chemical analyses, the mean of ~150 mg/L has been used for this review.
- A sediment TOC of 2% has been assumed, consistent with OECD formulated sediment specification.

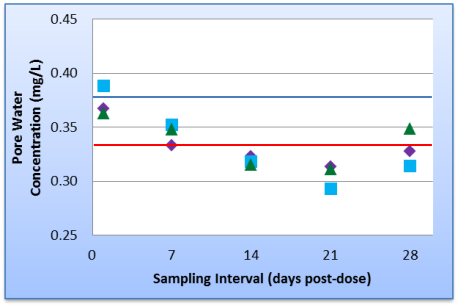


- The red line illustrates the point at which the measured concentration is equivalent to the estimated concentration at equilibration.
- Above the line, the measured concentration exceeded the estimated concentration, while below the line, the measured concentration did not meet the estimated equilibration concentration.
- Estimates of freely dissolved and total pore water concentrations are similar for low K_{oc} compounds, as shown by overlapping green and blue markers.
- As K_{oc} Increases, the measured concentration deviates further from the estimated freely dissolved concentration.
- For higher K_{oc} compounds, the green markers illustrate that estimating a total pore water concentration can establish a more applicable expectation for measured equilibration concentrations, as shown by the relative proximity to the red line.
- Circled data points show where measured and estimated concentrations (both freely dissolved and total) exceeded the compound-specific limit of solubility, establishing solubility as a confounding factor.

Application to Compound-Specific Data

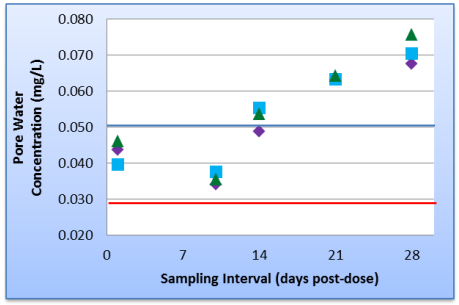
The plots below illustrate equilibration data for four compounds with varying physico-chemical attributes. The individual markers represent triplicate pore water analyses analyzed at each interval. The red line shows the freely dissolved estimated concentration based on sediment concentration and organic carbon content. The blue line shows the freely dissolved estimate adjusted to total concentration based on pore water organic carbon content.

Compound # 1 – K_{oc} = 700, 5.0 mg/kg dose



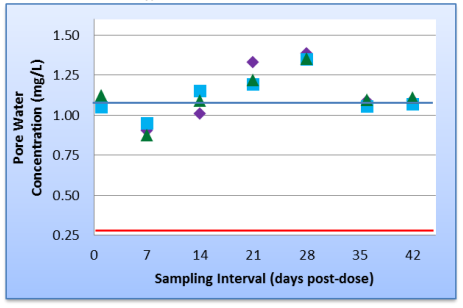
For compound #1, theoretical freely dissolved and total concentrations at equilibration do not differ much due to low K_{oc}. The trend in the data, as well as theoretical equilibration concentrations, establish that equilibration is achieved within a day of application.

Compound # 2 – K_{oc} = 5,400, 3.0 mg/kg dose



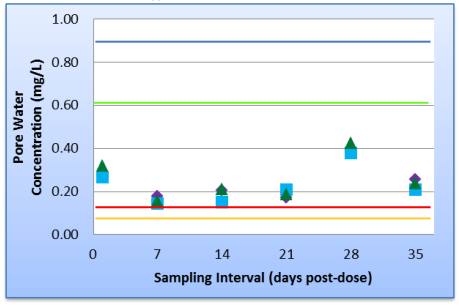
Compound #2 analytical data suggest that equilibration is never reached in 28 days. Applying a threshold of estimated total pore water concentration, equilibration is potentially achieved in ~ 14 days, as subsequent measurements far exceed any estimated expectation.

Compound # 3 – K_{oc} = 17,600, 100 mg/kg dose



Compound #3 analytical data start to illustrate the exaggeration of measured pore water concentrations relative to freely dissolved estimates for higher K_{oc} compounds. Analytical data again show no clear point of equilibration through 28 days, however, application of a theoretical total concentration at equilibration as a point of reference, suggests that equilibration is potentially achieved at 14 days.

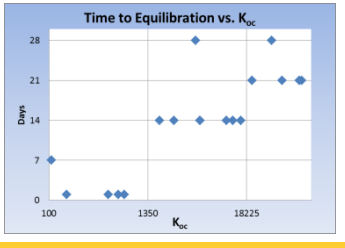
Compound # 4 – K_{oc} = 35,400, 100 mg/kg dose



For compound #4, estimated and measured concentrations all exceeded the limit of solubility (gold line). Application of EqP theory generates an estimated total concentration based on solubility (green line). This can provide a possible point of reference, suggesting that equilibrium was approached in 28 days. This scenario raises the question, how does solubility in pore water compare to other aqueous matrices?

Conclusions and Considerations

- Consideration of matrix TOC and EqP theory can provide useful context when evaluating the equilibration time of spiked sediments, however, it is also important to note that this review makes general assumptions regarding matrix TOC values based on historical data. A robust analysis would require interval specific analysis of pore water TOC.
- As partitioning coefficients are matrix specific, the generality of this evaluation must also be considered. Factors such as solubility and compound stability in sediment and pore water are also variables which may confound evaluation.
- Overall, data suggests that it may be possible to assume an appropriate equilibration period based on K_{oc} as illustrated in the figure to the right:



Thank you to the analytical chemistry and sediment toxicology teams of Smithers Viscient for their expertise and data generation.