

**DRAFT RSET ISSUE PAPER #19 – Testing Protocols Available For Laboratory Based Freshwater Bioaccumulation Testing Under RSET**

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**QUESTION/ISSUE: What are the available laboratory-based freshwater bioaccumulation testing protocols available and what are the known advantages and disadvantages of these protocols?**

**DISCUSSION:**

**Background:** The current Dredged Material Evaluation Framework (DMEF) for the Lower Columbia River Management Area (EPA/USACE, 1998a) contains guidance for laboratory based freshwater bioaccumulation testing in section 9.4 of the manual. Bioaccumulation testing is currently a Tier III requirement when there is reason to believe that specific chemicals of concern may be accumulating in target tissues at levels of concern.

The DMEF and the Inland Testing Manual (EPA/USACE, 1998b), recommends a 28-day laboratory bioaccumulation tests for assessing the potential for constituents to bioaccumulate. The Inland Testing Manual recommends the use of two bioaccumulation test species where possible representing two different trophic niches such as a suspension-feeding/filter-feeding and a burrowing deposit feeding organism. For marine/estuarine systems, the DMEF has established a set of two species to be tested; an adult bivalve (*Macoma nasuta*) and an adult polychaete (*Nereis virens*, *Nephtys*, or *Arenicola marina*). For freshwater systems, the DMEF recommends the use of the oligochaete *Lumbriculus variegatus* but does not specifically recommend a second freshwater bioaccumulation test species. The Inland Testing Manual (Table 12-1) presents a list of candidate laboratory bioaccumulation test species, however, there are only three listed as appropriate for freshwater sediments; *Lumbriculus variegatus*, the mayfly *Hexagenia limbata*, and the amphipod *Diporeia sp.* Of these three, only *Lumbriculus variegatus*, is commonly used for 28-day solid phase bioaccumulation testing for freshwater sediments. Ingersoll et. al., (EPA, 1998) has stated that one of the disadvantages for the use of mayflies and the amphipod *Diporeia sp.* as a laboratory test species is the difficulty in culturing these organisms. It should be noted that the recently published “Regional Implementation Manual for the evaluation of dredged Material Proposed for Disposal in New England Waters” (EPA/USACE, 2004) states that only one freshwater bioaccumulation test species is required for dredge material testing.

**Discussion:** The Bioaccumulation Subcommittee identified the need to summarize the current status of freshwater bioaccumulation testing protocols and also discuss the need and options available for the development of new freshwater test protocols and species.

*Lumbriculus variegatus* is the standard freshwater bioaccumulation testing organism recommended by the DMEF and Inland Testing manual but has limited tissue biomass available for analytical chemistry testing of tissues, which limits the types of chemical analysis that can be conducted on tissues at the conclusion of the standard 28-day laboratory bioaccumulation test.

In general, using existing EPA/ASTM protocols, the mean wet weight mass of tissue that can be collected from the replicate exposure chambers is approximately 8 to 9 grams. Depending on the nature and chemicals of interest in the test sediment, this tissue mass may be insufficient to be able to run complete analytical chemistry testing on more than one or two classes of compounds. For example, testing for PCBs/Pesticides, or semivolatile compounds, or metals each requires about four to six grams wet weight of tissue to provide an adequate amount of mass for chemical analyses. By reducing the available amount of tissue for chemical testing, the consequence can be that not all required analytes can be tested for, detection limits may become elevated due to insufficient tissue mass, and no extra tissue is available for secondary extraction and analysis if any QA/QC problems arise during the initial analysis.

There have been efforts to develop analytical methods that do not require as much tissue for analysis, but at this point in time, these methods are not provided by commercial analytical laboratories and it is unclear whether all the appropriate method development activities have been completed.

One alternative that has been explored in the freshwater systems of the Pacific Northwest (and in other areas) is the use of the bivalve *Corbicula fluminea* as a second laboratory bioassay species. The bivalve *Corbicula fluminea* is also a recommended species by this subcommittee for *in-situ* bioaccumulation testing (Salazar, 2004). The advantage for the use of this species is that the available tissue mass at the end of the laboratory exposure is much greater than that for *Lumbriculus*, about thirty to forty grams wet weight per replicate. *Corbicula fluminea* is a bivalve found throughout the freshwater systems in the Pacific Northwest (as well as the United States in general), therefore, there is an ecological relevance to its use in bioaccumulation testing. Hart Crowser (2002) conducted side-by-side testing of these two species in 28-day bioaccumulation tests from potential reference sediments collected in the Willamette River in Oregon (Hart Crowser, 2002).

While this study focused on sediments that contained very limited concentrations of bioaccumulative compounds, the study did come to the conclusion that *Corbicula* is a promising candidate for use as a second freshwater laboratory bioassay species and survived and were healthy after 28-days of exposure using standard EPA/ASTM protocols in fine-grained and medium-grained sediment. The USACE (2001) provides a list of publications that have also evaluated the use of *Corbicula* as a bioaccumulation test species under a variety of test protocols.

One of the concerns that has been expressed with the use of *Corbicula fluminea* is whether the uptake kinetics of this species is similar to *Lumbriculus variegatus*. Bivalves are able to conduct avoidance behavior in unsuitable habitats/situations by reducing their respiration

and filter feeding which would consequently reduce exposure to sediment-associated contaminants. It has yet to be determined whether this is a real phenomenon and if it is, whether this difference would have any significance in regulatory decision-making.

#### **REFERENCES:**

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#### **RECOMMENDATION:**

**1. Compile and Evaluate Existing Data on Bioaccumulation of Various Classes of Bioaccumulative Compounds by *Corbicula fluminea* and Compare with Results from *Lumbriculus variegatus* tests.** This evaluation will be helpful to determine any differences in bioaccumulation kinetics between the oligochaete and the bivalve and the magnitude of the difference if such a difference exists. This information can be used as the basis for determining whether any discovered differences between these two test species are significant or not for regulatory decision-making.

**2. Conduct Additional Bioaccumulation Testing Using *Corbicula* as Projects Allow.** By increasing the amount of data available on these two species, we should be able to have greater certainty to any decision RSET makes as to recommendations for their use.

**3. Follow-up on Methods Development for Analytical Techniques that Utilize Reduced Tissue Volumes.** This exercise will help RSET determine the advantages and trade-offs present with the current methods available to conduct tissue analysis using low tissue volumes.

**4. Coordinate with Other Researchers that are Exploring Related Issues.** Scientist at

the USACE Waterways Experiment Station and other research institutions have completed studies using *Corbicula* as a test bioaccumulation species. Speaking and coordinating with these researchers may provide additional insight on the appropriate use of *Corbicula* in freshwater bioaccumulation testing.

**5. Recommend that Two Species be Used for Freshwater Bioaccumulation Testing.**

Once sufficient method development has taken place for *Corbicula fluminea*, it is recommended, where possible, that two species be used for bioaccumulation testing. This is consistent with the Inland Testing Manual recommendations (EPA/USACE, 1998b) that two species be tested to cover the range of accumulation rates amongst test species and to be environmentally protective.

**PROPOSED LANGUAGE:** None yet available.

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