

## **Toxicology Branch Study Plan**

### **USGS Columbia Environmental Research Center (CERC)**

Study code: 08-20-17

Title: Toxicity of metal-contaminated sediments from the southeast Missouri lead mining district to freshwater mussels and amphipods

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### **Background**

The southeast Missouri (SEMO) mining district contains large chat piles and/or tailings impoundments that cover thousands of acres of land. Movement of tailings and associated metals from these sites has led to extensive contamination of aquatic sediments and biota in streams that drain these areas, especially the Big River and its tributaries, which drain the St. Francois County and Washington County sites. Concentrations of lead, cadmium, and zinc in sediments from Big River exceed probable effects concentration (McDonald et al. 2000) at sampling locations over 30 miles downstream from mining areas (MDNR 2003, Madden et al 2006). MDNR (2003) documented reductions in benthic invertebrate density and diversity below the St. Francois County site. Freshwater mussel populations in the Big River have decreased in recent years in a reach extending nearly to the confluence of the Meramec River, 90.5 miles downstream from the Site (Dave Mosby, USFWS, Columbia MO, unpublished data). The Meramec River downstream of the Big River supports some of the largest remaining

populations of the federally endangered pink mucket (*Lampsilis orbiculata*) and scaleshell (*Leptodea leptodon*), which may be threatened by contaminated sediment from the St. Francois and Washington County sites.

Until recent years, there was uncertainty associated with the reliability of toxicity data for freshwater mussels, because of their unique life history and because of limited expertise in laboratory culture of the sensitive early life stages, glochidia and early juveniles and because of a lack of standardization of methods (Ingersoll et al. 2006). However, recent collaborative efforts by CERC scientists and others scientists in government, academia, and industry have resulted in development of an ASTM standard method for conducting water-only laboratory toxic tests with glochidia and juvenile mussels (ASTM 2008a). Laboratory water-only toxicity tests have documented the high sensitivity of early life stages of mussels to the toxicity of several contaminants, including some metals (Wang et al. 2007a,b,c). Intra- and inter-laboratory toxicity studies have demonstrated relatively uniform sensitivity of different mussel taxa to aquatic contaminants, suggesting that testing conducted with readily available surrogate mussel species (e.g. fatmucket, *Lampsilis siliquoidea*) may adequately define likely responses of listed mussel species (Wang et al. 2007a,b,c). Recent studies at CERC have adapted the methods published by ASTM (2008a,b) to include in-situ testing and to laboratory tests with contaminated sediments (Ingersoll et al. 2008, Chris Ingersoll, USGS, Columbia, MO, unpublished data).

This study will conduct chronic toxicity tests with early life stages of freshwater mussels (*Lampsilis siliquoidea*, fatmucket) and amphipods (*Hyaella azteca*) exposed to metal-contaminated sediments collected downstream of the St. Francois County and Washington County sites in the SEMO mining district, with the goal of evaluating potential damages to populations of listed mussel species exposed to metal-contaminated sediment. In addition,

standardized sediment toxicity tests with the amphipod, *Hyaella azteca* (USEPA 2000; ASTM 2008b) to allow comparison of mussel toxicity data to previous studies of metal-contaminated sediments from other mining areas. Results of these toxicity tests will be evaluate relative to results of physical and chemical characterization of test sediments.

## **Methods**

### Site selection

Sediments will be collected from about 20 study sites to characterize sources and downstream extent of metal contamination and sediment toxicity associated with the St. Francois County and/or Washington County sites. These sites were selected from locations of ongoing mussel population studies (project lead: Andy Roberts, USFWS, Columbia, MO) and crayfish population and in-situ toxicity studies (project lead: Ann Allert, USGS, Columbia, MO). Study sites will include Big River and tributaries downstream of the mining areas, plus several reference sites without known upstream mining impacts (Table 1). Reference sites will be located in streams in of comparable size and similar physical-chemical characteristics (e.g., upper Big River, Bourbeuse River), to allow statistical comparisons of toxicity test results in metal-contaminated sediments to uncontaminated sediments with similar physical characteristics.

[**NOTE:** Sediment samples from a few additional sites that are part of the CERC/Missouri Department of Conservation crayfish studies, but not part of the sediment toxicity studies may be submitted by the Ecology Branch (Ann Allert) for chemical characterization. These samples will be collected, processed, and analyzed as described below, but will not be included in toxicity tests.]

### Sediment collection and characterization

Composite samples of stream sediments will be collected from depositional areas to represent fine sediments at each site. Table 1 is a list of sites to be sampled. If there are insufficient fine-grained sediments at some of these sites, alternate sites may be sampled. Surficial sediments (about the top 10 cm) will be collected from depositional areas within the wetted stream channel using PVC scoops (Ingersoll et al. 2008). Two five-gallon plastic buckets will be filled to about 2/3 volume with sediment to obtain about 20-L of sediment from each sampling site. If sediment samples contain substantial fractions of particles less than 2 mm diameter (i.e., greater than about 10% by volume), sediments will be wet-sieved through a 2-mm diameter stainless steel sieve in the field to remove coarse particles, using a minimum quantity of site water (Ingersoll et al. 2008). The resulting composite sediment samples will be stored in the dark at 4 °C in a refrigeration truck (in the field) and in a walk-in cooler (at CERC).

At CERC, all sediments from each site (up to three buckets per site) will be combined and homogenized using an electric drill and stainless steel auger. A portion of the homogenized composite sediment sample from each site will be wet-sieved, using minimum quantities of site water and (if necessary) test water, to obtain the fine sediments (250 to 500 mL of sediments <250- $\mu$ m particle diameter) needed for mussel toxicity tests and chemical characterization.

Sediments and pore water will be analyzed to characterize metal concentrations and other characteristics that may affect toxicity in accordance with methods summarized in Ingersoll et al. (2008). Composite sediment samples from each site will be characterized to determine particle size distribution, total organic carbon, and percent water. Samples of the composite samples and the sieved fine sediment samples from each site will be collected for analysis of total recoverable metals. Pore water extracted by centrifugation of composite samples from each site will be

analyzed for dissolved metals, dissolved organic carbon, major cations and anions, and routine water quality parameters. Metal bioavailability will be further characterized in sediments from the composite sediment samples that will be carried through the toxicity test in extra test beakers (chemistry beakers). Passive pore-water samplers ('peepers'; Brumbaugh et al. 2007) will be deployed in each chemistry beaker during the amphipod test and retrieved for metals analysis after a 7-d equilibration period. Sediments from these extra chemistry beakers will be collected at the time of peeper retrieval for analysis of acid-volatile sulfide (AVS) and simultaneously-extracted metals (SEM).

Total recoverable metals and metals in centrifuged pore water will be analyzed by semi-quantitative multi-element scans by ICPMS. SEM extracts and peeper samples will be analyzed for five metals (Cd, Cu, Ni, Pb, Zn) by quantitative ICPMS. Portions of each composite sample will also be collected and archived (frozen, in opaque glass containers) for analysis of persistent organic contaminants (polycyclic aromatic hydrocarbons, polychlorinated biphenyls, and organochlorine pesticides). These analyses may be conducted on samples from up to 10 sites, depending on the results of toxicity tests.

#### Sediment toxicity testing

Whole-sediment toxicity tests will be conducted using juvenile (2-4 months old) fatmucket mussels and juvenile (7-8 days old) amphipods. Mussel toxicity tests will be conducted with the fine (<250 um) size fraction of sediments using methods adapted from ASTM (2008a,b), USEPA (2000) as described in Ingersoll et al. (2008). Amphipod toxicity tests will be conducted with composite sediment according to published test methods (ASTM 2008b; USEPA 2000). Both tests will last 28 days, with endpoints of survival, growth, and biomass.

Growth of individual animals will be assessed by digital measurement of body length (amphipods) or shell diameter (mussels). Total biomass for each replicate will be determined using length-weight relationships (for amphipods) or direct weight measurements (mussels). A negative control sediment (from West Bearskin Lake, Minnesota) will be tested concurrently with field-collected sediments to characterize performance of test organisms relative to performance criteria established by ASTM (2008a,b) and USEPA (2000).

Toxicity tests will be conducted in temperature-controlled water baths (23 °C) with automated replacement of overlying water (Ingersoll et al. 1998). The overlying water used in the tests will be well water diluted with de-ionized water to a water hardness representative of sites in the Big River watershed (200 mg/L as CaCO<sub>3</sub>). Sediment and overlying water will be placed in exposure chambers with test water (under static conditions) for about 1 week before the start of the toxicity tests to allow re-equilibration of sediment and pore water (Ingersoll et al. 2008). Additional details of test conditions are provided in Table 2. Statistical analyses of toxicity data will be performed in accordance with guidance outlined by USEPA (2000) and ASTM (2008a,b).

## References

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Table 1. List of sampling sites for sediment toxicity, mussel population study, and crayfish studies in streams near the Southeast

Missouri lead mining district. TOX-ID refers to site IDs used by Toxicity Branch; ECO-ID lists site IDs used by Ecology Branch.

TOX-ID	ECO-ID	Site Description (with <u>short name</u> )	Tox- icity	Mus- sels	Cray fish	N Lat (deg)	N Lat (min)	W Long (deg)	W Long (min)
SEMO-1	Ref-1	Big R. above <u>Irondale</u>	x	x	x	37	49.142	90	42.467
SEMO-2		Big R. at MDC <u>Leadwood</u> access	x	x		37	52.137	90	35.067
SEMO-3	TH-1	Big R. at <u>Hwy 67</u>	x	x	x	37	53.387	90	30.629
SEMO-4	TH-2	Big R. at <u>Hwy K</u>	x	x	x	37	55.521	90	29.919
SEMO-5	TM-1	Big R. at <u>Cherokee Landing</u>	x	x	x	37	57.298	90	33.189
SEMO-6		Big R. at <u>Hwy E</u>	x	x		37	58.053	90	34.490
SEMO-7		Big R. at <u>Hwy CC</u>	x	x		38	2.715	90	37.283
SEMO-8		<u>Mineral Fork</u> Cr. near MDC Kingston access	x	x		38	5.724	90	42.711
SEMO-9	TM-2	Big R. at <u>Mammoth</u> MDC Access	x	x	x	38	7.278	90	40.548
SEMO-10		Big R. at <u>Brown's Ford</u> MDC Access	x	x		38	12.758	90	42.370
SEMO-11	TL-2	Big R. <u>above Cedar</u> Hill Dam	x		x	38	20.976	90	38.591
SEMO-12		Big R. <u>below Cedar</u> Hill Dam	x	x		38	20.973	90	38.726

Table 1. (continued)

TOX-ID	ECO-ID	Site Description and Access	Toxicity	Mussels	Crayfish	N Lat (d)	N Lat (m)	W Long (d)	W Long (m)
SEMO-13		Big R. <u>above Rockford</u> Beach dam	x			38	25.216	90	35.409
SEMO-14		Big R. <u>below Rockford</u> Beach dam	x	x		38	25.349	90	35.220
SEMO-15		Big R. at <u>Byrne's Mill</u> Dam	x			38	26.267	90	34.989
SEMO-17		Big R. at <u>Hwy W</u>	x	x		38	27.270	90	37.365
SEMO-18		Big R. <u>above confluence</u> w/ Meramec	x			38	28.125	90	37.420
SEMO-19		<u>Meramec R. upstream</u> ; MDC Pacific Palisades access	x			38	38.629	90	42.925
SEMO-20		<u>Meramec R. downstream</u> ; Rte. 66 State Park MDC access	x	x		38	30.127	90	35.500
SEMO-21		<u>Bourbeuse R.</u> near MDC Choteau access	x	x		38	24.161	90	54.564
SEMO-22	Ref-2	Big R. at <u>Hwy U</u>			x				
SEMO-23	TL-2	Big R. at <u>Washington</u> State Park			x				

Table 2. Summary of test conditions for whole-sediment toxicity tests with amphipods and mussels, based on USEPA (2000), ASTM (2008a,b), and Ingersoll et al. (2008).

<b>Parameter</b>	<b>Description</b>
Test type:	Whole-sediment exposures with water renewal
Test sediments	20 test sediments (Table 1) plus control (West Bearskin Lake; WB)
Temperature:	23±1 °C
Lighting	Ambient laboratory light; 16 hr light/8 hr dark
Test chamber:	300-ml beakers, with screened overflow
Sediment preparation:	Amphipod test: composite sediments (wet-sieved to <2 mm particle size in the field) Mussel test: fine fraction of composite sediments (wet-sieved to <250 µm in the laboratory)
Sediment volume:	100 ml, with 175 ml of overlying water
Water Renewal:	2 volumes/d, starting 24 hrs before start of test. Sediments and test water will be equilibrated under static conditions for 6-8 d before water replacement starts.
Age of organisms:	Amphipods, <i>Hyalella azteca</i> , about 7-d old; Mussels, <i>Lampsilis siliquoidea</i> (fatmucket), 2-4 months old
Organisms/beaker:	10
Number of replicates:	4 per sediment per species, plus one chamber (with sediment and amphipods) for chemical analyses
Feeding:	Amphipods: yeast-cereal leaves-trout food suspension (USEPA 2000), 1 mL/d (1.8 mg/d). Mussels: CERC instant algal mixture; 2 ml twice daily (Wang et al. 2007b, Ingersoll et al. 2008)
Aeration:	None
Test water:	Diluted well water (target hardness, 200 mg/L as CaCO <sub>3</sub> )
Test Duration:	28 d
Endpoints:	Survival, growth, and biomass. See Methods for details.
Test acceptability:	Performance-based criteria outlined in USEPA (2000), ASTM (2008a,b).

Table 3. Tasks and costs for toxicity testing with sediments from SEMO mining district

Tasks	Units	Unit cost (\$K)	Item Cost (\$K)	Subtotal (\$K)
<u>A. Sampling</u>				
Sediment collection and sieving	20	0.50	10.0	
Pore-water preparation (centrifugation)	20	0.25	5.0	
Sampling subtotal				15.0
<u>B. Toxicity Testing</u>				
Mussel culture	--	--	10.0	
Mussel sediment toxicity test	20	1.00	20.0	
Hyalella sediment toxicity test	20	1.00	20.0	
Toxicity testing subtotal				50.0
<u>C. Sediment characterization</u>				
Sediment characteristics (TOC, grain size)	20	0.25	5.0	
Porewater characteristics (WQ, ions, DOC)	20	0.15	3.0	
Sediment metals (total recoverable, 2 size fractions)	20	0.50	10.0	
Porewater metals (centrifuged)	20	0.25	5.0	
Sediment SEM/AVS (in test beaker)	20	0.25	5.0	
Peeper metals (chemistry beaker)	20	0.15	3.0	
Sediment PAHs, PCBs, pesticides	10	1.00	10.0	
Sediment characterization subtotal				41.0
<u>D. Report Preparation</u>				
USGS editorial and publication costs	--	--	--	15.0
<u>E. Indirect Costs</u>				
Direct Costs				121.0
Bureau charge (7%)				8.5
<b>Total Proposed Funding</b>				<b>\$129.5</b>