[Draft for Public Review]

Addendum to the Cherokee County, KS; Jasper and Newton Counties, MO; and Tar Creek, OK Natural Resource Damages Assessment Plans: Evaluating injury to mussels by metals in the Tri-State Mining District

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INTRODUCTION

The Tri-State Mining District (TSMD) is a historical lead and zinc mining area that consists of about 500 square miles and includes portions of Kansas, Missouri and Oklahoma (Figure 1; MacDonald et al. 2009). The TSMD includes four designated Superfund sites within two U.S. Environmental Protection Agency (USEPA) regions[1], three U.S. Fish and Wildlife Service (USFWS) regions[2], and three Trustee Councils[3]. The TSMD was one of the world's foremost lead and zinc mining areas, yielding about 460 million tons of ore between 1885 and 1970. The lead and zinc deposits within the TSMD were associated with the geologic region known as the Ozark Plateau. The ore deposits were typically accessed using underground mining methods

[1] USEPA Regions 6 and 7

[2] USFWS Regions 2, 3, and 6

[3] Cherokee County Trustee Council in Kansas includes the Department of the Interior (DOI) and State of Kansas; Jasper and Newton Trustee Council in Missouri includes the DOI and State of Missouri; and Tar Creek Trustee Council includes the DOI, State of Oklahoma, Cherokee Nation, Eastern Shawnee Tribe, Miami Tribe, Ottawa Tribe, Peoria Tribe, Seneca-Cayuga Nation, and the Wyandotte Nation.

and ore-concentration processes, resulting in the production of sand- and gravel-sized particles called chat (i.e., coarse tailings) and sand- and silt-sized particles called tailings. Further smelting and refining of these ore concentrates was conducted at various locations within the study area or elsewhere. Historical mining activities in the TSMD have resulted in contamination of surface water, groundwater, sediments, and soils in the Spring River and Neosho River basins by lead, zinc, and other heavy metals (MacDonald et al. 2009).

From 2003 to 2006, Angelo et al. (2007) investigated the status of mussel communities in the Spring River basin relative to metal contamination from the TSMD and reported that both mussel taxa richness and total mussel density decreased in stream reaches downstream of historical lead-zinc mining. Mussel taxa richness had significant negative correlations with concentrations of lead, zinc and cadmium in stream sediments. In 2007, the U.S Geological Survey (USGS) conducted an investigation to characterize sediment toxicity and sediment chemistry at 70 sites across the TSMD. The USGS study included laboratory toxicity tests with mussels (*Lampsilis siliquoidea*) and amphipods (*Hyalella azteca*) (Ingersoll et al. 2008, Besser et al. 2015). Mussels were less sensitive than amphipods in sediment toxicity tests, but toxic effects on both species increased with increasing metal concentrations in sediments. The results of both mussel community surveys and sediment toxicity testing indicated that metals in surface water, sediment, and pore water likely contributed to impacts on mussels and other benthic invertebrates inhabiting the TSMD (MacDonald et al. 2009). Results of an integrated laboratory and field study in the Southeast Missouri Lead Mining District demonstrated that toxic effects on mussels in sediment toxicity tests were associated with severe impacts in mussel communities in the field (Besser et al. 2015).

The present study is designed to maximize the concordance of metal exposure and toxic effects between mussels in laboratory toxicity tests and mussel communities in streams draining the TSMD. Collection of sediment and water for analysis and testing will occur at the same sites characterized by community and habitat surveys. Laboratory toxicity tests will expose juvenile mussels to field-collected sediments and to waterborne metal mixtures based on concentrations at these sites, both separately and in combined (sediment-plus-water) exposures. The sensitivity of juvenile mussels to metal toxicity in these tests will be increased

by starting with younger animals and by extending the exposure period threefold, compared to previous studies. Concordance of effects on mussels in the laboratory and in the field will be evaluated by comparing concentration-response models based on metal concentrations in sediment, water, and mussel tissue. The sensitive responses of juvenile mussels in long-term laboratory toxicity tests with a TSMD field based water-plus-sediment exposure regime should correspond closely to mussel community responses documented by quantitative field surveys at sites in the Spring River drainage.

RATIONALE AND SCOPE OF INVESTIGATION

The goals of this investigation are to characterize toxicity thresholds for metal injury to mussels based on exposure to metals in water and in sediment. This characterization will be based on: (1) analysis of metals in water and sediment at selected sites in the TSMD, (2) laboratory toxicity tests with mussels and amphipods to evaluate long-term toxicity of metals (Zn, Pb, Cd) in water and sediment from the TSMD, separately and in combination; and (3) quantitative mussel community surveys at the same sites sampled for water and sediment chemistry and toxicity testing. This study has the following key components:

1. Mussel toxicity tests will start with small (1-mm shell diameter) juveniles and have a (12 week) exposure period.
2. Toxicity tests will include metal exposure from both field-collected sediment and from metal mixtures in overlying water.
3. Toxicity data from 42 day amphipod study tests will provide a direct comparison with results of previous (2007) whole sediment toxicity tests, and allow an assessment of the additional contribution of metal exposure via overlying water.
4. Sites for sediment collection for toxicity testing and mussel community surveys will be co-located to compare mussel responses from the laboratory and in the field.
5. Reference sites will be selected and characterized to ensure that they represent physical habitat characteristics of sites affected by mining.
6. Mussel metal exposure in both laboratory and field studies will be based on analyses of metals in sediment, pore water, overlying water, and tissues.

This investigation will involve coordination of sampling efforts by personnel from USGS (Columbia Environmental Research Center and USFWS; their contractor, Ecological Specialists Inc. (ESI); and other cooperators. The project will be organized into a series of tasks and work elements to be completed between Spring 2016 and Winter 2018. The components of this investigation are summarized below and in Tables 1 and 2, with detailed descriptions and Appendices 1-8.

DESCRIPTION OF TASKS AND WORK ELEMENTS

Task 1. Reconnaissance sampling and site selection.

1A. Sampling plan and pre-sampling logistics (Spring 2016). The process of selecting 25 primary sampling sites will begin with development of a list of about 40 candidate sites. The list of 40 sites will be developed based on published sediment chemistry and toxicity data (e.g. Ingersoll et al. 2008, MacDonald et al. 2009, Besser et al. 2015) and mussel community data from Angelo et al. (2007) and recent data from Missouri Department of Conservation and Kansas Department of Wildlife, Parks, and Tourism. In addition, a Geographic Information Systems (GIS) project will delineate historically stable stream reaches that are most likely to support populations of long-lived freshwater mussels.

1B. Reconnaissance sampling (Spring-Summer 2016). Reconnaissance sampling will take place at about 40 candidate sites. Each site will be characterized for important physical features (e.g., stream order, gradient, and channel stability) using GIS. The suitability of each candidate site as mussel habitat will be assessed using a mussel habitat checklist. The habitat checklist will include factors such as distance to bluffs or bedrock exposures, geomorphic stream features (e.g. riffles, pools, bars, terraces, and/or islands), features that indicate stream stability (e.g. evidence of channel migration compared to historic maps, incised channels, cut banks, etc.), distance from the mouth of the stream, degree of sediment embeddedness, general observations of sediment grain-size, woody debris, condition of riparian corridor, presence or absence of mussels (including Asian clams), and distance to sediment sampling locations. The exact means to record and quantify these factors on a checklist will be determined with preliminary site visits and may vary due to the diversity of stream habitats within the scope of this study (e.g. prairie and Ozark streams, and a broad range of stream order). The status of the

mussel community at sites with suitable habitat will be assessed by qualitative sampling (e.g., timed searches). Methods for the reconnaissance mussel community and habitat survey are presented in Appendices 1 and 5. Samples of sediment, stream water, and pore water will also be collected during the reconnaissance sampling. Samples of bed sediments will be collected with a polyvinyl chloride (PVC) scoop (used in previous TSMD sediment study; Ingersoll et al. 2008, Besser et al. 2015) to evaluate the availability of sediments in sand, silt, and clay size fractions ("bulk sediments"; <2 mm particle diameter) suitable for sediment toxicity testing, and to collect samples for a preliminary characterization of metal concentrations in bulk sediments by X-ray fluorescence spectroscopy (XRF). Detailed methods for collection and XRF analysis of sediment samples are presented in Appendix 2. Samples of stream water (subsurface grabs) and of sediment pore water (push-point sampling) will be collected for analysis of water quality and metals concentrations as described below (Elements 2A and 4A) and in Appendix 3.

1C. Selection of primary study sites (Summer 2016). The 25 primary study sites will be selected based on information from the reconnaissance sampling, including qualitative mussel surveys, sediment availability, and sediment metal concentrations by XRF. All primary study sites should include suitable mussel habitat, based on the mussel habitat criteria in Element 1B and should have sufficient quantities of fine streambed sediments nearby (e.g., within 200 meters) to allow assessment of sediment toxicity in the lab. Within these constraints, site selections will seek to include broad geographic coverage of the TSMD watershed as well as a broad range of metal contamination. Up to 20% of sites (5 of 25) should represent reference conditions, with sediment metals concentrations less than probable effect concentrations (i.e., less than 459 ug/g zinc, 128 ug/g lead, and 4.98 ug/g cadmium; MacDonald et al. 2000, Ingersoll et al. 2001) and habitat characteristics that are comparable to non-reference sites. Due to extensive contamination of downstream reaches of the Spring and Neosho rivers, reference sites representing downstream sites may be selected from streams outside the Spring and Neosho watersheds. Another 20% of sites will be selected from locations that have sediment metal concentrations greater than 20 percent effect thresholds (T20s) defined by MacDonald et al (2009) for the Spring and Neosho rivers watersheds, i.e., zinc greater than 2949 ug/g, lead

greater than 219 ug/g, and/or cadmium greater than 17 ug/g. Whether or not these high-metal sites support live mussels, they will have habitat characteristics that are consistent with the occurrence of mussel communities. The remaining sites will be selected to maximize geographic coverage of the mining-affected reaches of the Spring and Neosho river drainages, and to represent a range of sediment metal contamination between reference and high-metal sites.

Task 2. Field sampling of water, sediment, and tissue (Summer 2016-Summer 2017).

2A. Water and pore water sampling (Summer and Fall 2016). Sampling of stream water and in-situ sampling of pore water at the reconnaissance sites and primary sites will characterize metal concentrations and water quality parameters affecting metal bioavailability. Samples of stream water will be collected with subsurface grabs, and composite samples of pore water will be collected in-situ in or near mussel beds using push-point samplers (Zimmerman et al. 2005; Appendix 3). The first set of water samples will be collected during the reconnaissance sampling in early summer 2016 (40 sites) and the second set of water samples will be collected during the low flow conditions in late summer or fall 2016 (25 sites). Metal concentrations from these samples will provide information on spatial and seasonal variation in metal concentrations and water quality to characterize waterborne metal exposure to mussels in the field. These data will be used to establish metal concentration ranges and ratios of metal mixtures for water-only toxicity tests to be conducted in winter 2017 (Task 3B).

2B. Sediment sampling (Summer 2017). Sediment for use in laboratory toxicity tests with sediment-only and sediment-water exposures will be collected at the 25 primary sites in Summer 2017. Methods for collecting sediments will closely follow those used for the previous TSMD sediment study (Ingersoll et al. 2008, Besser et al. 2015; Appendix 4). Sediments will be extracted from depositional areas with a 3-or 4-inch diameter PVC scoop and will be wet-sieved through a wash bucket equipped with 2-mm stainless steel mesh to sand-sized and smaller particles (<2 mm) for toxicity testing. Sediments will be sealed in polyethylene buckets with stream water and air, and then held in a refrigerated trailer until transport to the USGS laboratory (Columbia Environmental Research Center, Columbia MO) for sediment toxicity testing.

2C. Tissue sampling (Summer 2017). Samples of live Asian clam, *Corbicula fluminea,* will also be collected from each primary site during the quantitative mussel survey. Composite samples from each site will be analyzed for tissue metal concentrations as a surrogate for site-specific metal exposure of freshwater mussels (Angelo et al. 2007) for comparison to tissue metal concentrations of juvenile mussels from laboratory sediment toxicity tests (Task 4D).

Task 3. Mussel community and habitat characterization (Spring/Summer 2017).

3A. Quantitative mussel community survey. Surveys of mussel communities will be conducted at 25 primary study sites as described in Appendix 5. At each site, mussels will be collected from randomly-selected quarter-square meter quadrats (up to 100 quadrats per site). These surveys will allow characterization of taxa richness, age/size distribution, and areal density of live mussels, and will provide information on the occurrence of dead and sub-fossil shells.

3B. Mussel habitat assessment. Habitat will be characterized at 25 primary sites, as described in Appendices 1 and 5. Habitat characterization will include both a GIS-based evaluation of channel stability at the watershed scale and characterization of site microhabitat-scale features (e.g., pebble counts). Data from mussel habitat assessment will document similarities and differences in physical habitat between reference and metal impact sites, and will document the relative contribution of habitat characteristics versus metal exposure in determining mussel community status.

Task 4. Toxicity testing.

4A. Mussel culture. Gravid adult female fatmucket mussels (*Lampsilis siliquoidea*) will be collected from a stream in Boone County, Missouri in the fall of 2016 and spring of 2017 and held in the USGS laboratory at reduced temperatures to delay the release of larvae. In advance of planned toxicity tests, mussel brood stock will be transferred to the laboratory of Chris Barnhart at Missouri State University, Springfield MO, for production of mussel larvae and their transformation to the juvenile stage (Barnhart 2006). Newly-transformed juvenile mussels will

be shipped back to the USGS laboratory and reared to the appropriate age for testing (about 1 mm shell diameter; 1-2 months after transformation).

4B. Water-only toxicity testing with metal mixtures (Winter-Spring 2017). Long-term water-only toxicity tests with juvenile mussels (84 d; survival, growth and biomass endpoints; Wang et al. 2015) and amphipods, *Hyalella azteca* (42 d; survival, growth, biomass and reproduction endpoints; USEPA 2000) will characterize toxicity of three different aqueous metal exposure regimes (Appendix 6). Each exposure regime will consist of different aqueous metal mixtures and/or different water qualities, selected based on the data from field sampling of TSMD sites to represent the range of geographic and seasonal variations of aqueous metal exposure across the TSMD. Water-only toxicity tests will be conducted in proportional diluters which each deliver a series of 5 two-fold dilutions (plus a control). These dilution series will allow estimation of threshold concentrations for toxicity of aqueous metal mixtures in TSMD stream water or pore water. These water-only toxicity thresholds will be the basis for the aqueous exposure regimes used for sediment-water testing (task 3C).

4C. Sediment toxicity testing (Summer-Fall 2017**).** Long-term mussel tests (84-d; Kunz et al. 2014) and long-term amphipod tests (42-d; USEPA 2000) will be conducted with bulk sediments (<2 mm) from the 25 primary sampling sites, tested with clean overlying water. Methods for these tests are described in detail in Appendix 7. The goal of these tests is to determine the contribution of sediment-associated metals to mussel toxicity. The sensitivity of mussels in 84-d mussel exposures will be compared to the 42-day amphipod tests and to the 28-day mussel tests previously conducted with TSMD sediments (Ingersoll et al. 2009, Besser et al 2015).

4D. Sediment-water toxicity testing (Summer-Fall 2017). Concurrent with the sediment toxicity tests described, long-term mussel tests (84 d) and amphipod tests (42 d) will be conducted to evaluate the combined effects of metal exposure via TSMD sediment plus a metal mixture in overlying water. The methods for these tests are described in Appendix 7. The overlying water of these tests will contain a metal mixture (Zn, Pb, Cd), added at a single concentration selected to represent a TSMD field based exposure regime in TSMD streams. The metal ratios and the overall metal concentrations in the overlying water will be selected based

on field sampling of stream water and pore water conducted in 2016, and on results of water-only toxicity tests (task 3B). The concentration of the overlying water metal mixture will be selected to represent typical concentrations at TSMD sites, but will not exceed concentrations estimated to have a 20% effect on mussel toxicity endpoints, based on results of water-only tests. The upper limit on the level of effect predicted from metals in overlying water is intended to ensure that there is a sufficient range for potential additional toxic effects to allow us to quantify the additional contribution of sediment metals to the combined toxicity from the water-sediment exposures. Thresholds for toxic effects of metals on juvenile mussels in these long-term tests will be estimated from metal concentrations in sediment and pore water and in mussel tissues sampled at the end of the exposure. Metal concentrations in tissues of exposed mussels will be compared to tissue metals data from *Corbicula* collected from the primary study sites (task 5A).

Task 5. Chemical analysis.

5A. Field-collected stream water and pore water (Summer and fall 2016). Stream water grab samples and push-point samples will be collected during the reconnaissance sampling (task 1B; 40 stream water and 40 pore water samples) and low flow sampling (25 stream water samples and 50 push-point samples) in 2016. The samples will be analyzed for filtered (<0.45 um) metal concentrations; major ions and dissolved organic carbon (DOC); and routine water quality parameters, including ammonia, using methods described in Appendix 8.

5B. Sediments for toxicity testing (summer 2017). Chemical analyses will characterize bulk (<2 mm) sediment and pore water from the 25 primary sites, using methods described in Appendix 8. Sediment will be analyzed for total recoverable (TR) metals, simultaneously extracted metals and acid volatile sulfide (SEM-AVS), total organic carbon (TOC), and particle size distribution. Pore-water samples will be collected from composite sediment samples and from toxicity test chambers using peeper diffusion samplers (Brumbaugh et al. 2007; Appendix 3) for analysis of metals, water quality parameters, major ions, and DOC. Portions of composite whole-sediment samples will be archived for potential analyses (up to 10 samples) for organic

contaminants such as pesticides, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls that may contribute to sediment toxicity.

Task 6. Data management and project report

6A. Database development and report preparation (2017 and 2018)**.** All data will be validated and entered into a Microsoft Access relational database. Statistical analyses will include comparisons of community and toxicity endpoints with reference sites, evaluation of associations among biological responses, metals, and environmental variables, and evaluation of thresholds for adverse effects, based on metal concentrations in sediment, water, and tissues. Data summaries and analyses will be presented in a peer-reviewed interpretive report (e.g., as a NRDAR administrative report or USGS report series) and/or scientific papers in peer-reviewed journals.

6B. Scaling injury, calculating damages, and restoration planning for mussels (2018). The data generated during the course of this investigation will provide a basis for determination of injury to mussels associated with exposure to metals in the TSMD. Concentration-response models (CRMs) and injury thresholds for mussels inhabiting the TSMD will help estimate spatial extent of benthic habitats with mussels injury, to support a habitat-equivalency analysis (HEA). Habitat characterization and mussel community surveys will define baseline conditions, so injury to mussels will be expressed in terms of area with reduced mussel productivity.

QUALITY ASSURANCE

All data collected for this study will be subject to routine quality assurance procedures. Procedures applicable to each phase of this study (mussel community survey; field sampling of water, sediment and tissue; laboratory toxicity testing; and chemical analysis) are indicated in table 3.These procedures are described in greater detail in the appendices.

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Table 1. Summary of tasks and work elements.

WORK ELEMENT	SUB-ELEMENT	DESCRIPTION
Task 1. Reconnaissance and Site Selection		
1A. Pre-sampling Logistics	Identify 40 candidate sites	Mussel/chemistry/toxicity data; access
1B. Site Reconnaissance	Site description	GIS; morphometry, flow
	Mussel habitat checklist	[PENDING]
	Qualitative mussel survey	Timed searches
	Sediment reconnaissance	Sediment grab (XRF; weight percent <2 mm)
	Water reconnaissance	Water grab; push-point (see 2A)
1C. Site selection	25 primary sites, including ~5 reference sites and ~ 5 high-metal sites	Based on mussels, habitat, XRF, stream order, drainage
Task 2. Water and Sediment Sampling		
2A. Water sampling	Surface water samples	40 recon sites +25 primary sites (1/site)
	Push-point samples	40 + 25 sites; metals (3/site), ions & DOC (1 composite/site)
2B. Sediment sampling	Sediment for toxicity tests	25 primary sites; collect with PVC scoop; sieve to <2 mm; need 8+ liters/site
2C. Tissue sampling	Tissues for metals analysis	1 composite sample of *Corbicula* per site
Task 3. Mussel Community Characterization		
3A. Habitat Assessment	40 reconnaissance sites	GIS (stability), habitat checklist
	25 primary sites	Pebble counts [and other parameters TBD]
3B. Quant. mussel survey	25 primary sites	Up to 100 X 0.25 m^2 quadrats per site
Task 4. Toxicity Testing		
4A. Mussel Culture	Two cohorts for testing (winter & summer 2017)	4000 & 8000 juveniles @ 1.5 mm shell diameter (~1-2 months)
4B. Water-only Tests	Amphipod toxicity tests (42-d)	Three metal mixtures (based on 2A), each tested at 5 dilutions (plus controls)

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Table 1 (continued)

WORK ELEMENT	SUB-ELEMENT	DESCRIPTION
Task 4. Toxicity Testing (continued)		
4C. Sediment Tests	Amphipod toxicity tests (42-d)	25 sediments (plus control sediment) tested with control water
	Mussel toxicity tests, (84-d)	
4D. Sediment-water tests	Amphipod toxicity tests (42-d)	25 sediments (plus control sediment) , plus 1 metal mixture in water (based on 3B)
	Mussel toxicity tests (84-d)	
	Mussel tissue analysis (84-d)	1 composite sample of *Lampsilis* per sediment
Task 5. Chemical Analyses		
5A. Field water and sediment	Stream water	Metals, WQ, ions, DOC (40+25 samples)
	Push-point	Metals (40 composites +75 samples); Ions, DOC: (40 + 25 samples)
	Sediment	XRF (40 samples)
5B. Laboratory pore water	Bulk sediment (peepers)	Metals, ions, DOC; 25 sites X 2 samples
	Exposure beakers	Metals (Zn, Pb, Cd): 2 tests X 25 sites X 3
5C. Sediment Chemistry	Sediment	TR metals, TOC, PSA: 25 sites SEM- AVS: 2 tests X 25 sites X 3 reps
	Sediment (Archived)	Pesticides, PAH, PCBs (up to 10 sediments)
5D. Test water chemistry	Water-only tests	3 tests X 6 conc. X 6 samples X 3 metals?
	Sediment-only tests	25 sediments X 3 samples X 3 metals?
	Sediment-water tests	25 sediments X 6 samples X 3 metals?
	Water quality	Temperature, D.O., ammonia, etc.
5E. Tissue chemistry	Tissue metals: *Corbicula* (field); *Lampsilis* (lab)	Metals (Zn, Pb, Cd): 2 species X 25 sites

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Table 2. Generalized timeline for TSMD mussel injury characterization studies.

Work Element	2016				2017				2018			
	Wint	Spr	Summ	Fall	Wint	Spr	Summ	Fall	Wint	Spr	Summ	Fall
1A. Prepare sampling plan	X	X										
1B. Reconnaissance sampling		X	X									
2A. Water sampling			X	X								
2B. Sediment sampling						X	X					
3A. Habitat assessment		X	X			X	X					
3B. Quantitative mussel survey						X	X					
4A. Mussel culture				X	X	X	X					
4B. Water-only testing					X	X						
4C. Sediment testing							X	X				
4D. Sediment-water testing							X	X				
5A. Field sample analysis			X	X								
5B. Whole-sediment analysis						X	X	X				
5C. Lab pore water analysis						X	X	X				
6A. Data validation and reporting				X	X	X	X	X	X	X	X	X

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Table 3. Summary of quality assurance procedures

QA Procedure	Mussel community	Water-sediment-tissue sampling	Toxicity testing	Chemical analysis
SOPs and/or study plans	X	X	X	X
Data sheets	X	X	X	X
Chain of custody forms	X	X	X	X
Photo documentation	X	X		
Verify mussel IDs	X			
Verify handwritten data	X	X	X	X
Reference toxicant tests			X	
Control sediment and water			X	
Test organism archives			X	
Field and laboratory blanks		X		X
Field and laboratory duplicates		X	X	X
Standard reference materials				X
Laboratory spikes				X

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LIST OF APPENDICES

1. Reconnaissance mussel and habitat survey
2. Preliminary analysis of metals in sediments using XRF
3. Field and laboratory sampling of pore water
4. Sediment sampling for toxicity testing and chemical analysis
5. Quantitative mussel community survey
6. Long-term water-only toxicity testing with mussels and amphipods
7. Long-term sediment and sediment-water toxicity testing with mussels and amphipods
8. Chemical analysis of stream water, overlying water, and sediment
9. Field Data sheets
10. Chain of Custody sheet
11. Photograph documentation protocol

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Appendix 1: Reconnaissance mussel and habitat survey

Approximately 40 candidate sites will be investigated during the reconnaissance mussel survey. Sites will be chosen using various criteria, including channel stability (as indicated by concordance between recent aerial photos and 1960s-era topographic maps) and existing mussel and habitat data obtained from the Missouri Department of Conservation, Oklahoma Department of Wildlife Conservation, Kansas Department of Wildlife and Parks, and Angelo et al. (2007). At each of the candidate sites, the stream reach will be searched for an existing mussel community and/or suitable habitat. Determination of suitable habitat will be based on the mussel habitat checklist developed by biologists from USFWS, USGS, and ESI based on the information below. The area of suitable habitat and/or the extent of the mussel community will be delineated with GPS and habitat characteristics will be recorded. The mussel community, if present, will be qualitatively searched to document species composition and relative abundance. Sufficient detail of the site will be noted to evaluate and select the 25 primary sampling sites for further quantitative assessment. Field data sheets and digital photographs of the sites will be saved as part of the permanent project file.

The majority of mussel species in the Spring and Neosho river basins require permanent, flowing water above stable, gravel-dominated substrates with a component of finer grained particles (e.g., sand). In stream reaches that meet these minimum, fundamental requirements, mussels can form dense, multi-species aggregations, generally termed mussel beds. The mussel sampling will encompass a stratified approach that avoids pools and targets the riverine community. In Phase I, the Spring and Neosho rivers and their tributaries will be qualitatively surveyed by qualified malacologists to identify potential mussel habitat and delineate sites for quantitative sampling in Phase II. The following criteria will determine if the reach met minimum habitat requirements for best supporting a mussel community: 1) a stable river channel; 2) permanent, flowing water (i.e. riffles, runs, glides), 3) stable, generally compact, consolidated substrate that provides fine-grained materials ranging in size from 2-8mm in diameter (i.e., sand and fine gravel) and 4) presence of native unionid mussels or Corbicula detected within one person-hour of searching.

If any site meets those criteria or if the malacologist(s) believes the site is suitable regardless of the criteria, a second assessment will be conducted to attempt to characterize the quality of those site selection criteria. This approach will help to compare/rank/differentiate sites to ensure Phase II quantitative assessments include the highest quality sites in this watershed. The criteria evaluation will be a compilation of degrees of channel stability, bank erosion, presence of macrophytic vegetation, stream bed stability, substrate composition, permanence of water, adequate flow, and level of mussel presence.

Appendix 2. Preliminary analysis of metals in sediments using XRF

During the reconnaissance sampling, the availability of sand-sized and smaller sediments (<2 mm size fraction) in accessible (wadeable) habitats (<1 m depth) will be assessed at each site. At each site, sediment (about 0.5 L) from several potential sampling areas will be collected following methods in previous studies at TSMD (Ingersoll et al. 2009, Besser et al. 2015) with PVC scoop sampler, composited, homogenized, and stored in a plastic container.

Following EPA method 6200 (USEPA 2007a), the reconnaissance sediment samples will be dried in the laboratory at room temperature or in a low temperature oven (<60 C). After the total dry weight of the composite sample is determined, the sample will be crushed and passed through a 2 mm stainless steel sieve, and the weight of the <2 mm fraction (and the weight percent of the <2 mm fraction) will be determined. A portion of each <2 mm sediment sample will be placed in a plastic bag for XRF analysis.

Sediment samples will be analyzed by XRF for one minute by placing the instrument window directly against a portion of the bag that is in full contact with the sediment. Three separate readings will be collected for each sample; sediment bags may be hand-kneaded between readings to ensure sample homogeneity, or the XRF may be directed against three separate bag sites, since the XRF penetration depth is only a few millimeters or less. These results will be recorded on a datasheet and stored electronically in a spreadsheet.

A suite of calibration verification check samples will be used to check the accuracy of the XRF instrument and to assess the stability and consistency of the analysis for the analytes of interest. Check samples will be analyzed at the beginning of each working day, during active sample analyses, and at the end of each working day. The measured value for each target analyte should be within ±20 percent (%D) of the true value for the calibration verification check to be acceptable. If a measured value falls outside this range, then the check sample should be reanalyzed. If the value continues to fall outside the acceptance range, the instrument should be recalibrated, and the batch of samples analyzed before the unacceptable calibration verification check will be reanalyzed. In addition, 5% of sediment samples analyzed by XRF will be subsequently re-analyzed to determine total-recoverable metals by ICP-MS, following methods described in Appendix 8. In addition, field data sheets will be saved as part of the permanent project file

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Appendix 3. Field and laboratory sampling of sediment pore water.

Pore water samples for analysis of metals and other water quality parameters will be collected both in the field and in the laboratory. Field sampling of in-situ pore water from streambeds will be conducted using push-point samplers. Samples will be stored temporarily in a cooler then transported to the USGS laboratory in Columbia, MO for sample processing and distribution for analysis. Transfer of the pore water samples from the field to USGS laboratory and from USGS to other laboratories will be documented on a field chain-of-custody form. Documentation of field operations associated with sample collection will be recorded on field sampling sheets. Digital photographs of the sample sites and reference objects will be saved as part of the permanent project file. Sampling of pore waters in the laboratory will be conducted with "peeper" diffusion samplers deployed in composite sediment samples before testing, and in extra test beakers during toxicity tests.

A.3.1. Push-point samplers:

Push-point samplers (also known as drive points; Zimmerman et al. 2005) are a suction-based in-situ method for obtaining sediment pore water in shallow water (depths to about 1.5 m). The push-point method is most applicable in sediments dominated by small cobble and sand substrates because sediments dominated by silt and clay size particles may clog the probe inlets before a suitable sample volume can be collected. USGS has obtained 3-ft (45-cm) and 6-ft (91-cm) samplers, fabricated by M.H.E. Products (East Tawas, MI). These samplers are made of high molybdenum #316 stainless steel (SS), with a sampling area perforated with 6.35 mm o.d. x ~5 mm i.d. slots that spans 2 cm, with 3 alternating slots on each side of tube. An inner solid SS support rod is supplied with each tube which may be used to provide internal tube support when driving the probe downward into compacted sediments or soils. However, use of the inner rod is typically not needed in most sediment substrates, particularly when sampling from relatively shallow sediment depths. This procedure assumes that the inner support rod is not required for sampler insertion. These push-point samplers are primarily designed for sampling volatile and semi-volatile organic compounds (VOCs) from soil or shallow sediment; however, USGS has successfully modified them for use in sampling sediment pore waters for metals.

Before collection of samples for metals analysis, push-point samplers are first cleaned at the laboratory using acetone-soaked swabs, then treated inside and out with a **thin** coating of high-purity silicone vacuum grease. Note that care must be taken not to clog the sampler slots with grease during application. Clean samplers are stored, with the inner SS support rod in place, inside a protective casing; and the sampler tips are further protected inside a short piece of Tygon tubing for storage. Just prior to field sampling, the support rod is removed, and each sampler is fitted internally (typically in the field) with a pre-made section of concentric, 2-component plastic tubing consisting of 3/16 inch (4.76 mm) o.d. x 9/64 inch (3.572 mm) i.d.

polypropylene (PP) tubing with a slightly longer section of 1/8 inch (3.175 mm) o.d. x 2 mm i.d., fluorinated ethylene propylene (FEP) tubing inserted inside. The inside end of the FEP tubing is flared with a heat-flaring tool so that once the 2-component plastic tubing is installed into the push-point housing, the inner FEP tubing can be pulled back from the opposite end to make the bottom end seat flush with the outer PP tubing. Notably, the inner FEP tubing from which the pore water is drawn has a "void" volume of 1 mL per foot of length. Thus, a 3-ft length has a void volume of 3 mL, and a 6-ft length has a void volume of 6 mL, which is the volume that should be drawn and discarded when sample is first drawn from the respective push point tube. A one-way valve placed in-line between the syringe and the end of the FEP tubing is used to keep the pore water from draining from the push-point during multiple draws per deployment, or "stab", location. When on-site, a polycarbonate (PC) spacer plate is attached to the bottom section of the tube to ensure the sampling probe is inserted to a consistent depth (typically 4-6 cm) into the sediment each time a sample is collected, and to minimize intrusion of surface water into the pore water while it is drawn. A PP mesh "sock" pre-filter is fitted onto the push-point tip before pressing it into the sediment. A single push point sampler, inner concentric tube assembly, and polypropylene pre-filter "sock" can be used for multiple stabs/reaches within a single site, but a clean sampler and new inner assembly and sock should be used at each site. Figure 1 show each end of a completed assembly ready for sampling.

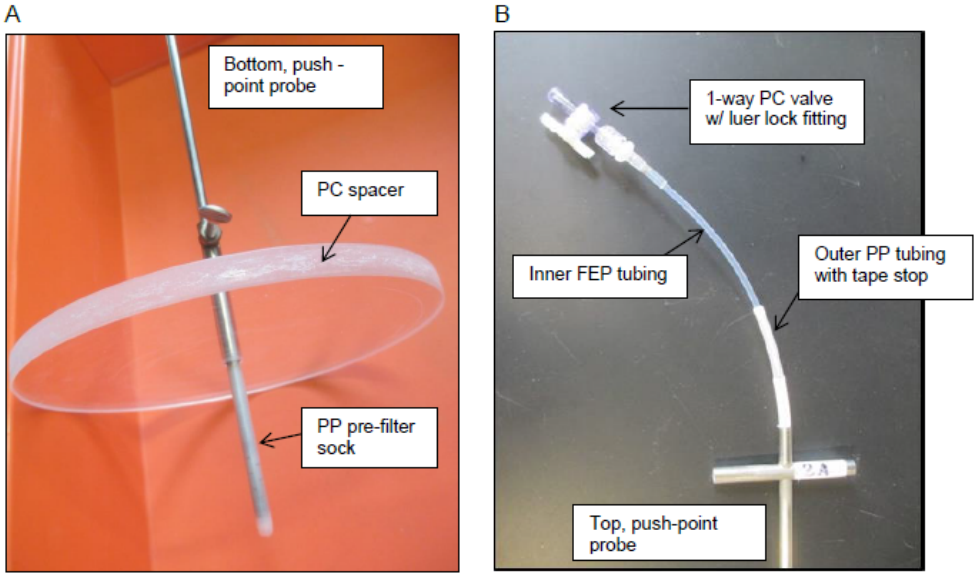

Figure 1. A push-point sampler equipped (A) at the bottom with a PP pre-filter "sock" and PC spacer, and (B) at the top with the concentric tubing assembly inserted to the tape stop, and fitted with a luer lock valve.

Sampling shallow pore water with 3-ft push-point tube. This procedure describes collecting 3 subsamples (or metals, anions, and DOC) from a single push-point deployment (or "stab"). Multiple deployments may be necessary to generate composite samples with sufficient volume

for these analyses. It is recommended that no more than 60 mL of pore water be collected from each stab location. It should be noted that sample bottles for metals and anions will be supplied filled with high purity water; these should be emptied completely just prior to sample collection. Further, it is recommended that the site be sampled downstream to upstream if multiple reaches per site are to be evaluated.

1. Remove a push-point sampler from the protective case. Remove the inner SS support rod and temporarily place it back in the protective case.
2. Remove the short piece of protective Tygon tubing from the probe tip. Attach the PC spacer plate followed by a PP sock prefilter to the end of the probe. Adjust the spacer plate so that it is situated immediately above the sock and tighten the thumbscrew to lock it in place (Figure 1a). With this arrangement, the pore water sample will be drawn from a sediment depth range of about 4-6 cm. Socks should be inspected for damage between stab locations, and replaced as needed.
3. Insert a clean concentric PP-FEP tubing assembly into the push-point housing. The taped end with the valve attached will remain above the top of the SS probe after insertion. Insert the **non**-taped end completely until the taped section serves as a "stop" so that the tubing is not inserted too far (bottom end is not situated below the beginning of the slots). Gently pull the inner FEP tubing out of the probe until the flared end is flush with the outer PP tubing.
4. Press the push-point tube assembly into the sediment until the spacer plate is flush with the bottom. It should stay upright without providing any support. If it doesn't, remove and try reinserting at a nearby location. Gently pull back on end of the inner FEP tubing to ensure the flared bottom end inside the SS tube is still flush with the end of the outer PP tubing.
5. Attach a 24-mL, all polypropylene syringe to the 1-way PC lure-lock valve at the upper end of the FEP sample tubing (Figure 1b).
6. Slowly draw about 3 mL into the syringe. Close the valve, remove the syringe from the luer-lock valve and discard the 3 mL.
7. Reattach the syringe, open the valve, and slowly draw about 22 mL of pore water for metals. Close the valve and remove the syringe from the valve. If substantial air bubbles are drawn through the push-point during this process, it might be necessary to close the valve, expel the air from the syringe, and redraw additional water to obtain the required volume.
8. Attach a filter cartridge designated for metals (PP pre-filter + polyethersulfone, PES filter). Dispense 2 mL through the filter to waste, and dispense remaining 20 mL into a pre-cleaned LDPE bottle designated for metals. **Discard filter.**
9. Reattach the syringe, open the valve, and slowly draw about 17 mL of pore water for anions. Close the valve and remove the syringe from the valve.

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10. Attach a filter cartridge designated for anions (PP pre-filter + PES filter; same type as for metals). Dispense 2 mL through the filter to waste, then dispense remaining 15 mL into a HDPE bottle for anions. **Discard filter**.
11. Reattach the syringe and slowly draw about 22 mL of pore water for DOC. Close the valve and remove the syringe from the valve.
12. Attach a filter cartridge designated for DOC (GDX glass pre-filter + PES filter). Dispense 2 mL through the filter to waste, then dispense the remaining 20 mL into an amber glass vial for DOC. **Discard filter**.
13. Remove the push-point probe assembly from the sediment. Draw up about 20 mL of surface water with the syringe (no filter) for use in back-flushing the probe before conducting the next "stab". Attach water-filled syringe and back-flush the probe with the tip elevated above the water surface so that the flushing can be seen. Repeat until flushing can be done with little resistance and the water appears to be flowing equally well from all six slots and/or with even distribution from the sock. Note that it is critical to collect the samples in the prescribed order (metals/cations, anions, then DOC), since the probability of the plastic syringe releasing carbon is reduced after the two "rinses" with the metals/cations and anions portions of the pore water.

A.3.2. Peeper diffusion samplers:

Peepers (Carignan et al. 1985) are passive diffusion samplers that have been used by USGS to sample pore-water metals, major ions, and DOC from sediment samples both in the lab and in the field (Brumbaugh et al. 2007, Besser *et al.* 2011). Peepers are constructed from acid-cleaned plastic snap-cap vials with a hole punched in the cap. The vial is filled with de-ionized de-oxygenated water and a 0.45 um PES filter membrane is secured under the cap. Acid-cleaned vials are submerged in de-ionized, de-oxygenated water as the membrane is secured under the cap. Peepers are then deployed by submerging them in sediment for the typical deployment of 7-10 days, which allows dissolved constituents inside the vial to equilibrate with pore water outside the membrane. Previous USGS investigations indicated that the ionic composition of the liquid inside of either small (2.9 mL) or large (24 L) peeper was greater than 95% equilibrated with that of the surrounding water within 5 to 6 days at 20 °C. After the equilibration period, the peeper is removed, rinsed with deionized water, and the cap is carefully opened to allow access to the sample.

Both large and small peepers will be used to sample pore waters in this project. Pore water for analysis of metals, major ions, and DOC will be sampled from large volumes of composite sediments by deploying 3 or more large (24-mL) peepers. Large peepers will be buried about 3 cm deep in the sediment container with the membrane facing towards the center of the

sediment container. Peepers will be deployed for 7-10 days and samples will be collected when sediments are removed to start toxicity tests.

Pore water for in-situ sampling of metals during toxicity tests will be sampled from test chambers using 2.9-mL peeper samplers that will be buried between 1 and 2 cm below the sediment surface on day 14 of toxicity tests. Note that the prescribed burying depths vary between the large and small peeper sizes simply due to the need to bury the entire peeper face diameter in the sediment. Separate "chemistry" beakers containing test sediments and test organisms will be used for these deployments. Peepers will be removed after equilibration for 7-10 days, and at the same time sediment from each of these chemistry beakers will be transferred to a 60 mL amber glass jar will be collected for analysis of simultaneously extracted metals and acid-volatile sulfide (SEM-AVS).

Peeper preparation: Using a hole-punch or other suitable tool, a hole is punched in the center of each vial cap (with the cap still attached to the vial) such that only a small lip remains around the outer edge of the vial cap to support and secure the PES membrane. The hole diameters are approximately 6 mm for the 2.9-mL peepers, and 14 mm for the 24-mL peepers. Punched vials are cleaned by soaking overnight (with occasional agitation to wet all vial surfaces) in a plastic bottle containing 4M HNO3, 2M HCl, followed by sonication at 50 °C for 20 minutes. The vials are triple rinsed with de-ionized (DI) water then stored submerged in DI water until further peeper preparation. To prepare the peepers, a small acid-cleaned plastic tub is half-filled with freshly de-oxygenated, de-ionized water (DODI water) and up to 24 cleaned and punched vials (caps in the open position) are submerged in it (use a fresh batch of DODI water for each 24 vials). Wearing suitably clean and pre-DI-rinsed waterproof gloves, a submerged vial is grasped with the cap open and held with its top edge just at the water surface. A PES filter membrane is then placed over it (aligned with minimal overlap near the hinged area of the vial) and the perforated cap is carefully closed to seal the membrane. Excess membrane material on the outside is torn away and discarded, leaving a small portion opposite the hinge to facilitate complete removal of the membrane when opening. Once seated, the membrane is inspected for rupture and the peeper is inverted above the water to check for leaks. The peeper is inverted only momentarily, otherwise water droplets may begin to seep through the membrane. A correctly filled and sealed peeper has no air bubbles inside. A small nylon cable tie is strapped around the vial to aid retrieval and the finished peeper is transferred to a wide-mouth, 500 mL acid-cleaned, polypropylene bottle containing DODI water and one "scavenger" peeper (identifiable by the absence of a nylon cable tie) that contains about 100 mg of 100-mesh size, metal-chelating resin. After all peepers have been added, the storage bottle is "topped off" with DODI water, then capped tightly and placed in a refrigerator. Each set of peepers will include three peepers for use as blanks and the one scavenger peeper. Peepers will

be stored in this manner for up to 4 weeks before use; however, the surrounding water will be replaced and de-oxygenated once again 24 to 48 hours before deployment.

Peeper deployment/retrieval: Peepers will be transported to testing area in the storage bottle (filled with DODI water and the peeper containing the metal-chelating resin). Deployment will be performed in one of two ways depending on the sediment density and grain size. For most samples, the peeper will be forced down into the sediment with a plastic or titanium spatula and plastic (hemostat type) forceps and "back-filled" with a small amount of sediment. If difficulty is encountered with that approach, (e.g., for dense or highly granulated sediments), a small trench will be dug into the sediment by inserting a titanium spatula and prying it sideways. Regardless of the burial method, the bottom (closed end) of the peeper is situated next to the wall of the test chamber and the membrane end situated toward the center so as to maximize the sediment volume "seen" by the membrane face. After seven days in the sediment, a rack containing eight sediment test chambers containing the peepers will be transported to a lab bench for transfer of liquid contents of each peeper as described below.

Following the removal of the peepers, a sample of the corresponding sediment will be transferred from the beaker to a 60-mL glass jar fitted with a TFE-lined screw cap for analysis of SEM-AVS. For this, any overlying water will be decanted from the beaker and the sediment will be briefly stirred using an acid-cleaned plastic v-scoop. Using the scoop, a 60-mL sub-sample is transferred to the jar (filling the jar completely). A laboratory tissue is used to remove any excess sediment or grit from the threads of the jar. The cap is secured tightly and sealed with vinyl tape, and the jar is stored in a refrigerator.

Transfer of peeper contents: Each peeper vial will be removed from the sediment by grasping the tag end of the nylon cable tie with the plastic forceps. The peeper will be gently agitated in the overlying test water to remove loosely adhering sediment particles. Next, the peeper is rinsed using a stream of DI water directed tangentially to the lid and membrane until all visible particles are displaced. Finally, the peeper vial is blotted dry using a laboratory tissue. The membrane/perforated cap assembly is carefully opened with a DI-rinsed, gloved hand by grasping the protruding edge of membrane in conjunction with the edge of the cap. It will be opened carefully to prevent the membrane from falling into the liquid inside the vial. The liquid inside will be transferred to an acid-cleaned LDPE bottle (30 mL volume for small peepers or 60 mL volume for large peepers) using a pre-rinsed disposable polyethylene pipet. Just before use, each (new) pipet will be cleaned by drawing a small volume of high-purity 1% nitric acid, inverting to contact the inside of the bulb and all inner surfaces, and then expelling the acid to waste. The inversion rinse procedure will then be repeated using high purity water. Using the cleaned pipet, the liquid inside the peeper is transferred to an opened, tared LDPE bottle. After

complete transfer of the liquid from the peeper, approximately 2.5 mL of high purity 1% (v/v) nitric acid for the 2.9-mL peeper, or approximately 24 mL of 1 % nitric acid for the 24-mL peeper is added to the peeper vial using a squirt bottle. It is important not to fill the peeper vial to the brim with the high purity 1 % nitric acid rinse solution or the peeper liquid may become contaminated with particulate matter. This rinse liquid is then transferred to the receiving LDPE bottle using the cleaned disposable pipet. For 2.9-mL peepers, the partially diluted peeper liquid is then diluted to a final volume of 29 mL (29.2 g) with 1 % nitric acid, to result in an overall sample dilution factor of 10, for ICP-MS analysis. For 24-mL peepers, no additional dilution will be used, resulting in an overall dilution factor of 2, for ICP-MS analysis. The transfer procedure will be repeated for each peeper using a new, cleaned disposable pipet. A maximum of eight disposable pipets may be cleaned using the same portions of pipet rinse solutions (1 % nitric acid and high purity water) before these rinse solutions must be replaced.

Blanks: Three blank peepers will be prepared and stored in the bottle containing DODI water and metal-chelating resin for every 18 to 20 test peepers. These will be prepared, rinsed, opened, and transferred at the same time and in the same manner as each group of sediment-deployed peepers.

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Appendix 4. Sediment sampling for toxicity testing and chemical analysis

A 4.1. Sediment volume requirements. About 5 L of material is required to support chemical analyses of the sediment and pore water. An additional 5 L of sediment is needed for toxicity testing with amphipods and mussels. Therefore, a minimum of about 10 L of sediment is required for each sampling location. Whenever possible, it would be preferable to collect about 15 L (about 4 gallons) of sediment from each site, to allow for settling of sieved sediments and to leave about 2 L of volume for site water and airspace in each 5-gallon sample bucket.

A 4.2. Sampling equipment. Sediment sampling crews will assemble the following equipment:

- 3" diameter PVC (schedule 40) scoop and replacement sampling heads, constructed by USGS personnel;
- Wildco wash bucket (US10; equipped with 2-mm heavy-duty stainless steel mesh (part 190 J11 2000);
- Two 5 gallon plastic buckets with lids for each sampling site that will be visited that day.

Other treatment and supplies needed include: Map of the study area, showing study sites; field data collection sheets and clipboard; Latex or rubber gloves; Digital camera; Global positioning system (GPS) unit; Alconox solution in a 1L squeeze bottle; Nylon scrub brushes; and Plastic bags;

A 4.3. Sediment sampling. At sites that are deemed suitable to sample, the sampling crew will use the hand-held GPS unit to locate the middle of the site and survey the site, recording data on the field data collection sheet. Sample buckets will be roughly one-third filled with site water and the wash bucket will then be placed inside one of the five-gallon sample collection buckets. The PVC sediment sampler will be used to collect sediment from the top 3 inches of the streambed. The scoop should be slowly pushed along the sediment surface with an attempt to minimize loss of fine grained particles. Contents of the scoop are dumped into the wash bucket and the bucket is agitated to sieve the finer grained material into the surrounding five gallon sample collection bucket. Material not passing through the 2-mm mesh at the bottom of the wash bucket will be returned to the stream. Repeat this process until each sample bucket contains about 8 L of sieved sediment. The sieved sediment and stream water should be consolidated into a single bucket, leaving 1-2 L of airspace, and bucket is sealed. After sampling a site, decontaminate the sampling equipment by cleaning with river water and Alconox solution, using a brush if necessary, then rinsing with river water and air drying. Store cleaned equipment sealed in clean plastic bags or wrap, or aluminum foil.

Samples will be stored temporarily in a refrigerated truck then transported to the USGS laboratory in Columbia, MO for sample processing and distribution for analysis. Transfer of

these sediment samples from the field to USGS laboratory and from USGS to other laboratories will be documented on a field chain-of-custody form. Documentation of field operations associated with sample collection will be recorded on field sampling sheets. Digital photographs of the sample sites and reference objects will be saved as part of the permanent project file.

A 4.4. Sample Handling and Processing. Sediment samples will be held at 4°C until processing begins. It is anticipated that the samples will be processed during the week immediately following sediment collection. Sample processing will involve decanting excess overlying water, homogenization, and sub-sampling to facilitate physical, chemical, and biological characterization. More specifically, excess overlying water (over about 5% of the sediment volume) will be decanted and sediment will be homogenized with a hand held drill and stainless steel auger. Care will be taken to fully homogenize the sediment sample by mixing at low speeds to minimize entrainment of air in the sample. Samples will be considered homogenized when the entire sample has achieved a uniform texture, color, and consistency. Following homogenization, sub-samples will be obtained for toxicity testing and physical and chemical characterization as described in Appendices 6-8.

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Appendix 5: Quantitative mussel community survey

Approximately 25 primary study sites will be selected for quantitative mussel community and habitat assessment. These sites will be selected based on results from the reconnaissance mussel survey, preliminary characterization of sediment metal concentrations by XRF, and assessment of the availability of sufficient quantities of sand-sized and finer-grained sediment (<2 mm diameter; "bulk sediments").

At each primary study site, mussels will be collected quantitatively using up to 100 quarter-meter (0.25 m^2) quadrat samples randomly placed within the delineated suitable mussel habitat area and/or mussel community extent. Each quadrat sample will be excavated to a depth of approximately 15 cm, and all live mussels will be identified to species, measured (length in mm), and aged (external annuli count). Fresh-dead shells will be identified and counted and weathered-dead and sub-fossil shells will be noted as present. Quantitative sampling will allow characterization of taxa richness, density, and age and size distribution.

Live Asiatic clams (*Corbicula sp.*) encountered during the quantitative sampling (or, if necessary, during additional sampling) will be collected and placed on ice to obtain a sufficient sample of soft tissue from each site for determination of tissue metals concentrations, using methods similar to those described by Angelo et al. (2007). *Corbicula* samples will be frozen at the end of each sampling day and shipped to the USGS laboratory for analysis.

At the time of quantitative surveys, micro-scale habitat features (depth and pebble count at each sample point) will be recorded to compare physical habitat characteristics between reference and impact sites and to document the influence of habitat characteristics on mussel community status. Documentation of field operations associated with sample collection will be recorded on field sampling sheets. Digital photographs of the sample sites and reference objects will be saved as part of the permanent project file.

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Appendix 6. Long-term water-only toxicity tests with mussels and amphipods

Overview: Up to three water-only toxicity tests will be conducted with mussels and amphipods exposed to different metal mixtures and/or different water chemistries, to represent the range of metal exposures of stream biota in the TSMD. Selection of metal mixtures and/or water chemistries for testing will be made based on results from field sampling of stream water and pore water (task 2A). Mussel tests will be based on ASTM method 2455 (ASTM 2015b), but the duration of tests will be extended from 28-days to 84-days (12 weeks). Amphipod tests will be based on the standard 42-day whole sediment test protocol (USEPA 2000, ASTM method 1706 (ASTM 2015a), with diets and test acceptability criteria modified to optimize performance in water-only test conditions.

A6.1. Apparatus and test conditions. Chronic toxicity tests will be conducted in up to three intermittent-flow proportional diluters. Stock solutions will be delivered with each cycle of each diluter by a Hamilton syringe pump (Hamilton, Reno, NV, USA), with each diluter delivering five exposure concentrations (with a dilution factor of 2) plus a control. Each one-liter delivery of water from the diluter is split into 8 equal volumes delivered to 8 300-ml replicate glass beakers in a polypropylene splitter box (0.125 mL/beaker /cycle). Each beaker sees a thin layer of silica sand (about 5 mL of <500-µm particles; Granusil #4030, Unimin Corporation, New Canaan, CT). The diluter cycles once every hour in the mussel test (15 volume-additions/d), and once every 4 hours in the amphipod test (2 volume additions/d). Excess water drains from each beaker via a 2.5-cm hole in the side covered with 50-mesh (279-µm width opening) stainless-steel screen. The exposure system will be maintained at 23 °C, with ambient laboratory light (~500 lux) and a 16 h light:8 h dark photoperiod.

At the start of the chronic testing, ten mussels or ten amphipods will be impartially transferred into each replicate beaker and an additional four replicates (10 mussels or 20 amphipods per replicate) will be collected for measurements of starting length and dry weight. Dry weights of mussels and amphipods will be determined after drying at about 60°C for 24 h. Mussels will be fed 2 ml of algal mixture (510 nl cell volume/ml) delivered automatically by a pump into each replicate beaker with each cycling of diluter every hour during the first 4-week exposure.

Afterwards, the feeding level will be increased about 30-50% every 4 weeks depending on algal consumption by mussels. Algal mixture will be prepared daily by adding 1 ml of Nannochloropsis concentrate and 2 ml of Shellfish Diet concentrate (Reed Mariculture, Campbell, CA) into 1.8 L of water (Wang et al. 2007). Algal mixture will be prepared daily before the morning feeding and kept in a refrigerator at <4°C for the feeding in the rest of the day. The stocks of algal mixture will be maintained in aerated containers at 4°C and changed daily. Algal concentrations in the stock and in selected treatments right before and after feeding will be measured periodically using algal cell counter. The amphipods will be fed once daily with

ramped Diatom (*Thalassiosira weissflogii*, 1200TM, ReedMariculture, Campbell, CA) and ramped Tetramin diet (Tetramin as suspended flakes). Feeding rate will be increased over exposure time (details in Table 4).

Water quality (dissolved oxygen, pH, conductivity, hardness, alkalinity, and ammonia) will be measured in the chronic exposures in the control, medium, and high concentrations every other week. Water samples for analysis of metals (zinc, lead, cadmium) will be collected at the start of the exposure and every other week from each of the six exposure concentrations. Composite water samples for analysis of DOC, major cations (calcium, potassium, magnesium, and sodium) and major anions (fluoride, nitrate, nitrite, and sulfate) will be collected from the exposure chambers from the control and medium concentrations at Weeks 0, 2, 4, and 6 in amphipod exposure and at Weeks 0, 2, 4, 8, 12 in the mussel exposures.

A 6.2. Test endpoints. Every two weeks, surviving mussels and amphipods in each replicate beaker will be transferred into a 200-ml glass dishes containing about 100 ml of the test solution from the replicate beaker for survival determination. Mussels with or without foot or shell movement, with a gaped shell containing swollen or decomposed tissue, or with empty shell will be counted and recorded. Mussels with a gaped shell containing swollen or decomposed tissue and with empty shell are classified as mortalities and removed from the test. Surviving mussels and amphipods will be transferred back to their replicate beaker after rinsing the beaker thoroughly with test water (contained in a plastic squirt bottle) and the addition of new sand to the beakers. The sand will be held in the control water for 24 hours before placement in exposure beakers.

Four mussel replicates per treatment will be destructively sampled at day 28 for determination of survival, weight and dry weight, and biomass. Mussels will be collected by passing sediments from test beakers through U.S. standard size #40 mesh sieve (425-µm opening). The remaining four replicates will be destructively sampled at day 84 for determination of weight and biomass. Surviving mussels will be frozen at the end of the test prior to determination of individual wet and dry weights. Individual dry weights will be summed to estimate biomass for each replicate.

Amphipod reproduction will be recorded weekly starting at day 28. Surviving amphipods at day 42 will be preserved in 8% sugar formalin for subsequent determination of number of males and females in each replicate and for determination of individual lengths, and mean dry weight for each replicate

Table A 6-1. Summary of test conditions for conducting water-only chronic toxicity test with fatmucket (*Lampsilis siliquoidea*) and amphipod (*Hyalella azteca*) in basic accordance with ASTM International (2015a,b) and USEPA (2000).

Parameter	Conditions
1. Test chemical:	Zinc-lead-cadmium mixtures (up to 3 combinations of metal mixture and water quality
2. Test type:	Daily renewal, water-only exposure
3. Test Duration:	Mussel: 12 weeks Amphipod: 6 weeks
4. Temperature:	23°C
5. Light quality:	Ambient laboratory light
6. Light intensity:	About 500 lux
7. Photoperiod:	16L:8D
8. Test chamber size:	300-ml glass beaker
9. Test solution volume:	200 ml containing about 5 ml of sand (Granusil #4030, Unimin Corporation sieved to <500-µm particles)
10. Renewal of water and substrate	Mussel: 125 ml every hour (about 15 volumes/day) Amphipod: 125 ml every 4 hours (about 4 volumes/d) Every 4 weeks, surviving mussels and amphipods in each replicate beaker will be counted and transferred into a new beaker with new sand
11. Age of test organism:	Mussel: About 6 weeks after transformation Amphipod: 7-day old
12. Organisms/test chamber:	10
13. Replicates/concentration:	8 (4 replicates destructively sampled at week 4 and at week 12 for mussels and 8 replicates destructively sampled at week 6 for amphipods)
14. Feeding:	Mussels: 2 ml of algal mixture delivered automatically into each beaker every hour; feeding level will be increased about 30 to 50% every 4 weeks Amphipods: Diatoms (*Thalassiosira weissflogii* [ramped from 0.5 mg/beaker-d (week 1) to 2.5 mg/beaker-d (week 6)] and Tetramin: [ramped from 0.25 mg/beaker-day (week 1) to 2.5 mg/d (week 6)]
15. Aeration:	None
16. Dilution water:	To be determined (based on 2016 sampling in TSMD)
17. Dilution factor:	2

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18. Test concentrations:	TBD
19. Chemical residues:	Metals in filtered (<0.45 µm) water: 4 times and the amphipod test and 6 times in the mussel test s
20. Water quality:	Dissolved oxygen, pH, and ammonia, weekly. Composite samples for DOC, major ions
21. Endpoint:	Mussels: survival, growth and biomass 4 and 8 weeks Amphipod: growth, biomass reproduction, 6 weeks
22. Test acceptability criteria :	Mussel: ≥80% control survival in 4-week exposure. Amphipod: 42-d control survival ≥80%, weight ≥0.4 mg/individual, and reproduction ≥6.0 young/female (updated TACs; Chris Ingersoll, USGS, unpublished data)

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Appendix 7. Long-term sediment and sediment-water testing with mussels and amphipods

Overview: Two sets of long-term toxicity tests will be conducted with 25 field-collected sediments, with and without added metals and overlying water, to evaluate the contributions of sediment and water to overall exposure to toxic metals to benthic organisms in the TSMD.

> **Test 1** will be an exposure to TSMD sediments, with clean overlying water.
>
> **Test 2** will consist of the TSMD sediment exposure, plus exposure a mixture of metals in water based on results of TSMD field sampling (Task 2A) and water only toxicity testing (Task 3B).

A 7.1. Test conditions. Methods for whole-sediment toxicity tests are based on standard or published methods for conducting acute and chronic whole-sediment toxicity tests with freshwater invertebrates (e.g., ASTM 2015a,b; USEPA 2000; Besser *et al.* 2015; Wang *et al.* 2007, 2013). The duration of sediment tests with freshwater mussels will be extended from 4 weeks to 12 weeks, following methods presented by Kunz et al. (2014).

Test conditions and procedures for sediment toxicity tests with and without metals and overlying water are summarized in Table A 7.1. Test apparatus and test conditions for whole sediment tests are similar to those described for water only tests in (Appendix 6) exposure beakers will contain 100 mL of sediment and 175 mL of overlying water, with excess water overflowing through a screened notch at the lip of each beaker. Beakers in Test 1 will receive clean test water, and beakers in Test 2 will receive an aqueous mixture of zinc, lead, and cadmium at a single concentration, representing a 10% to 20% effect concentration determined from water only tests. The solution will be prepared in a large reservoir and delivered via the diluter's delivery lines. Test 2 will include two control treatments: a sediment control that will receive the same metal mixture as other treatments, and a water control that will receive both clean sediment and clean overlying water.

About one week before the start of the sediment tests (i.e., about Day -7), each sediment sample will be homogenized, placed into exposure beakers, and clean overlying water will be added. The sediments will be held under static conditions to allow the sediment samples to equilibrate with oxygenated overlying water before the start of the exposures (Ingersoll *et al.* 2008, Wang *et al.* 2013). Water delivery will be started on the exposure beakers on Day -1 and test organisms will be impartially transferred to the exposure beakers on Day 0. At the start of the sediment exposures, archived samples of each species, each consisting of four replicates of 10 organisms, will be collected for the following measurements of starting size: (1) amphipod length and dry weights estimated from length-weight regression; Ingersoll *et al.* 2008; (2) mussel dry weight, and AFDW. Amphipod samples are archived in a solution of 12% sugar and

8% formalin (80 mL concentrated formalin and 120 g sucrose in 1 L water) to preserve body dimensions before length measurements (ASTM 2015a). Mussel samples are archived in 8% formalin without sugar to remove attached microfauna and debris before weighing.

A 7.2. Test endpoints. After 4 weeks, mussels will be separated from sediment in each beaker and survivors recorded as described above, and 4 mussel replicates per treatment will be destructively sampled for determination of survival, weight and dry weight, and biomass. Surviving mussels in the 4 remaining replicates will then be transferred to clean beakers that had been refilled with 100 mL of fresh sediment and clean overlying water 24 hours previously, and returned to the exposure system. The remaining 4 replicates will be transferred to clean sediment in beakers again after 8 weeks, and will be destructively sampled at Week 12 for determination of weight, biomass and metal concentrations in soft tissues. All surviving mussels will be frozen at the end of the test prior to determination of individual wet weights. Selected individuals near the median weight for each replicate will be frozen to make composite samples from each exposure level for analysis of tissue metals. Remaining surviving mussels from each replicate will be used to determine the average dry mass content for each replicate, and the biomass of mussels of each replicate will be determined by summing the average estimated dry weight (wet weight X average dry mass content) of all surviving mussels in each replicate. Samples of soft tissues will be prepared for analysis using methods similar to those described by Angelo et al. (2007); tissue analyses will follow methods described in Appendix 8.

Sediment from amphipod test beakers will be sieved on Day 28 to isolate surviving amphipods and survivors from each replicate will be placed into water-only beakers containing clean water and 5 mL of quartz sand. The number of young in each replicate and survival of adult amphipods will be determined on Day 35 and Day 42. Surviving adult amphipods on Day 42 will be preserved in sugar-formalin for length measurement and for determination of the number of males and females in each replicate. This information is used to calculate the number of young produced per female per replicate from Day 28 to Day 42. The biomass of surviving amphipods from each replicate at the end of the exposures will be estimated as the sum of individual amphipod weights calculated from a length-weight regression.

Statistical analyses of survival, weight, biomass, reproduction, or emergence data will be performed in accordance with requirements outlined in USEPA (2000) and ASTM (2015a,b). Responses of animals in non-reference sediments will be evaluated by statistical comparison to responses in control sediments and reference sediments (e.g., Besser et al. 2015 Wang *et al.* 2013). Relationships between metal concentrations in sediment, water, and/or tissue and responses of mussels in laboratory tests or in the field will be examined using concentration-response models (e.g., Toxicity Relationship Analysis Program; Erickson et al. 2010).

Concentration-response models will be used to estimate concentrations of metals or metal mixtures that are associated with various levels of biological effects, (e.g. EC20, the concentration associated with 20% reduction in a specified effect such as survival or growth.)

A 7.3. Characterization of sediment, pore water, and overlying water**.** During toxicity tests, samples of whole sediment and pore water will be collected from separate replicate chemistry beakers. One separate replicate chemistry beaker containing sediment and test organisms will be set up for each of the 25 sediment treatments in the amphipod test and two replicate chemistry beakers will be set up for each of the 25 sediments in the mussel test

Peeper diffusion samplers (appendix 4) will be deployed in chemistry beakers on about day 10 (in both tests) and on day 70 (mussel test) to sample pore-water metals. After peeper samples are collected, sediments from chemistry beakers will be sampled for analysis of SEM-AVS.

During all tests, analyses of overlying water will include: temperature (daily); dissolved oxygen, pH, and conductivity (weekly); and ammonia, hardness and alkalinity (at the beginning and end of each test).

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Table A 7-1. Test conditions for conducting whole-sediment toxicity tests and sediment+water tests with amphipods, and mussels with TSMD sediments

Parameter	Description
1. Test description	**Test 1: TSMD sediment test with clean water;** **Test 2: TSMD sediment with TSMD metal mixture in water** Amphipod (*Hyalella azteca*) and mussels (*Lampsilis siliquoidea*) 25 TSMD sediments (TBD) plus Control(s) TBD
2. Temperature	23 (°C)
3. Light quality	Wide-spectrum fluorescent lights (about 500 lux, 16L:8D)
4. Test chamber	300-mL high-form lipless beaker
5. Sediment: Water	100 mL:15 mL
6. Water renewal	About 4 volume additions/d
7. Sediment renewal	Amphipods: none (transferred to water-only beaker at 4 wk) Mussels: rinse beaker, replace sediment at 4 and 8 wk
8. Age and size of organisms	Amphipods: About 7 d (mean dry wt. 0.02-0.035 mg) Mussels: About 2 months (mean length about 1.5 mm).
9. Organisms/chamber	10
10. Replicates/sediment	Amphipod, mussels: 8 replicates each. Chemistry: 3 replicates
11. Feeding	Amphipod: Ramped rations of Tetramin suspension and diatoms (*Thalassiosira weissflogii* 1200TM; Reed Mariculture, Inc. Campbell, CA). Mussel: 3 mL of non-viable algae twice daily. [1 mL *Nannochloropsis* and 2 mL Shellfish Diet in 1.8 L of water; Reed Mariculture, Campbell, CA.]
12. Aeration	None, unless dissolved oxygen in overlying water drops below 2.5 mg/L for amphipods or 4 mg/L for mussels (ASTM 2015b).
13. Overlying water	Sediment-only test: Control water TBD Sediment+water test: TSMD zinc-lead-cadmium mixture TBD
14. Chamber cleaning	Clean screens as needed
15. Overlying water quality	Temperature daily. Dissolved oxygen and ammonia weekly. Hardness, alkalinity, conductivity, pH and ammonia twice in amphipod test; 3 times in mussel a test.
16. Chemistry in test beakers.	Beaker pore water: peepers in chemistry beakers for a 7-d equilibration period (sampled day 28 and 56). Beaker whole sediment: SEM-AVS (days 28 and 56).
17. Endpoints	Mussel tests (84 d): survival, growth, biomass, metals in tissue.

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Parameter	Description
18. Endpoints (contd.)	Amphipod tests (42 d): survival, growth, biomass, reproduction
19. Test acceptability criteria (controls)	Mussels: day-28 survival at least 80% Amphipods: day-28 survival at least 80%; mean dry weight 0.5 mg; mean reproduction 5 young/female

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Appendix 8. Chemical analysis of water, pore water, and sediment

Overview: Stream water, pore water and overlying water from toxicity tests will be analyzed for metals, DOC, major ions, and other water quality parameters. Analyses of whole sediments collected for toxicity testing will include particle size analysis and determination of organic carbon, percent water, and total recoverable metals, with sampling occurring before the start of the exposures. Samples for SEM-AVS will be collected from chemistry beakers (extra beakers stocked and fed along with the toxicity test on Day 14 of the amphipod exposures).

A 8.1. Metals and major cations in waters. Concentrations of dissolved (filterable or dialyzable through a 0.45 um pore diameter) metals (Zn, Pb, Cd) and major cations (Ca, K, Mg, Na) in stream water, diluter overlying waters, and sediment pore waters (collected by grab, peeper, and/or push-point samplers) will be measured using a PerkinElmer ELAN DRC-e inductively-coupled plasma-mass spectrometer (ICP-MS). Peeper samples will be collected and processed as described in Appendix 4. Push-point samples will be collected according to the procedure given in Appendix 4, and upon transfer to USGS, will be preserved with a sufficient volume of sub-boiling distilled nitric acid to achieve a final matrix of 1 % (v/v) nitric acid. Stream water and overlying waters from toxicity tests will be similarly filtered with a 0.45 um filter disc (PES or PP/PES), and preserved by acidification with sub-boiling distilled nitric acid (1 % v/v final matrix). The ICP-MS method is similar to USEPA 6020B (2014). The ICP-MS is equipped with an autosampler/autodilutor, and a mixing tee for external addition of an internal standard solution. A minimum of three external calibration standards plus a calibration blank will be used to calibrate the instrument response, and established laboratory quality assurance/quality control (QA/QC) procedures (e.g., laboratory spikes, duplicates, control samples) will be used to verify instrument performance throughout the analyses.

A 8.2. Dissolved organic carbon and anions in water. Concentrations of major anions in stream water, diluter overlying waters, and sediment pore waters (collected by grab, peeper, and/or push-point samplers) will be measured in filtered (0.45 um PP/PES) samples using an ion chromatography system (Dionex ICS-1100) equipped with a Dionex Ionpac AS22 anion exchange column and a suppressed conductivity detector. A 4.5 mM sodium carbonate/1.4 mM sodium bicarbonate mobile phase will be used to elute the anions of interest. The ion chromatography method is similar to USEPA 9056A (USEPA 2007b). A minimum of three external calibration standards plus a calibration blank will be used to calibrate the instrument response, and continuing calibration blanks and standards, laboratory spikes, duplicates, and other laboratory QA/QC procedures will be used to verify instrument performance throughout the analyses. Anion samples will be stored in a laboratory refrigerator at 4 °C for no more than 30 days prior to analysis.

Concentrations of dissolved organic carbon (DOC) in stream water, diluter overlying waters, and sediment pore waters (collected by grab, peeper, and/or push-point samplers) will be filtered (0.45 um glass/PES) as described in Appendix 4, or by a vacuum filtration assembly (0.45 um PES), and dispensed into pre-cleaned low-TOC amber vials. The samples will be acidified with 9N high-purity sulfuric acid (BDH Aristar Ultra, VWR International, Radnor, PA) to pH 2 or less within 48 hours of receipt. Acidified samples will be refrigerated and held no longer than 28 days prior to DOC analysis. The DOC will be measured as non-purgeable organic carbon (NPOC) by high temperature combustion catalytic oxidation-nondispersive infrared spectroscopy using a total organic carbon analyzer (Model TOC-L CSH, Shimadzu Scientific Instruments, Inc., Columbia, MD). The method is similar to USEPA method 415.3 (USEPA 2009). Samples are air sparged to remove inorganic carbon species prior to the NPOC measurements. Instrument and method performance were verified using continuing calibration and blank verification standards, analytical spikes and duplicate analyses.

A 8.3. Total-recoverable metals in sediments. Total recoverable metal concentrations in whole-sediment samples will be analyzed by ICP-MS. As previously described, the ICP-MS method used by USGS is similar to USEPA method 6020B (USEPA 2014). The sediments will be microwave digested in a mixture of nitric and/or hydrochloric acids and/or hydrogen peroxide using a method similar to USEPA 3050B (1996). This method is a very strong acid digestion that will dissolve most elements that could become environmentally or biologically available, but will not release elements bound in silica structures. Typically, Zn, Pb, and Cd are fully recovered from sediment reference materials using this total recoverable method.

Simultaneously-Extracted Metals and Acid-volatile sulfide (SEM-AVS) in sediments. Measurements of SEM-AVS in sediment samples will be performed using a two-part process. First, the sediment will be mixed with 1 N hydrochloric acid (20:1 volume 1 N HCl to sediment wet weight) in a nitrogen-purged oxygen-free atmosphere to release sulfide as hydrogen sulfide gas. The hydrogen sulfide is then bubbled through a pH 12 antioxidant trapping solution to form free sulfide, which will be measured using a sulfide-specific electrode (Brumbaugh et al. 2011; USEPA 376.3 [1991]; Table A2.6). All reagents will be deoxygenated, and exposure to air will be minimized. Samples will be refrigerated and analyzed within 28 days of receipt. Any pourable liquid in the sediment samples will be decanted to waste when opening the sample jars, and the upper 1 cm of sediment in the jar will be removed and discarded. The remaining sediment sample will be gently stirred for a maximum of 1 minute to produce a uniform consistency before sampling. Quality control measures will include sulfide electrode initial and continuing calibration, laboratory spikes, and blanks.

After completion of the sulfide extraction, the sediment-HCl mixture will be allowed to settle for a maximum of 15 minutes, and then 40 mL of the extract will be filtered through a 0.45 um

PES membrane, diluted in nitric acid, and/or further digested, and/or volume-reduced as needed for ICP-MS analysis. The ICP-MS method has been previously described in Section A8.1. The SEM metals of interest may include one or more of the following: Ag, Cd, Cu, Pb, Ni, and Zn.

A 8.4. Total recoverable metals in mussel tissues. Mussel or surrogate mollusk tissues (e.g., *Corbicula*) collected for metals analyses will be lyophilized, homogenized, and microwave-digested in nitric acid and/or hydrogen peroxide prior to ICP-MS analyses. Some compositing may be required of individual animals to achieve approximately 100 mg of lyophilized tissue for analysis. The USGS digestion and analysis methods are similar to USEPA 3050B (USEPA 1996) and 6020B (USEPA 2014), respectively. As before, a minimum of three external calibration standards plus a calibration blank will be used to calibrate the instrument response, and established laboratory quality assurance/quality control (QA/QC) procedures (e.g., laboratory spikes, duplicates, control samples) will be used to verify instrument performance throughout the analyses.

A 8.5. Sediment TOC and particle size analyses**:** Total organic carbon content and particle size distribution of sediments will be determined using standard methods 4H2 and 3A1a, respectively, from the USDA Kellogg Soil Survey Laboratory (NRCS 2014). For particle size analysis, the sieved soil fraction (< 2 mm) is characterized for particle size distribution using a dispersion and suspension method. The sample is pretreated with peroxide and other reagents to remove organic matter and soluble salts, and then oven-dried to obtain an initial weight. The particles are then dispersed with a sodium hexametaphosphate solution and mechanically shaken to create a suspension. The sand fraction is removed from the suspension by wet sieving and then fractionated by dry sieving. The clay and fine silt fractions are determined using the suspension remaining from the wet sieving process. This suspension is diluted to 1 L in a sedimentation cylinder and stirred, and then 25-mL aliquots are removed with a pipette at calculated, predetermined intervals. The aliquots are dried at 110 °C and weighed. Coarse silt is the difference between 100% and the sum of the sand, clay, and fine silt percentages.

For carbon analysis, the sieved soil fraction (< 2 mm) is packed in foil, weighed, and analyzed for total C by an elemental analyzer. The elemental analyzer uses catalytic combustion in an oxygenated atmosphere and high temperature to liberate carbon as CO2, which is detected using a thermal conductivity detector. The inorganic carbon fraction is determined by treatment of the soil with hydrochloric acid and subsequent manometric measurement of the evolved CO2, while the organic carbon is estimated by difference of the total and inorganic carbon fractions.

Appendix 9 – Field Data Sheets

A9-1. Example Field data sheet for water sampling.

United States Department of the Interior
U.S.GEOLOGICAL SURVEY
Columbia Environmental Research Center
4200 New Haven Road
Columbia, MO 65201

SAMPLE COLLECTION & SHIPMENT RECORD FOR SAMPLES TRANSMITTED TO USGS-CERC

PROJECT LEADER OR SAMPLER:_____

PROJECT TITLE OR DESCRIPTION:_____

TRANSMISSION DATE:_____

TRANSMITTED FROM (name):_____

TRANSMITTED TO (name):_____

BRIEF DESCRIPTION OF SAMPLE SET:_____

_____

TOTAL NUMBER OF SAMPLES IN SHIPMENT:_____NUMBER OF CONTAINERS:_____

SAMPLE TYPE(S):_____

_____

COLLECTED BY:_____

DATE(S) COLLECTED:_____

COLLECTION PERMIT ID:(if applicable)_____

GENERAL COLLECTION LOCATION(S):_____

_____

HOW ARE SAMPLES PACKAGED?_____

_____

WHO PACKAGED SAMPLES?_____

METHOD(S) OF PRESERVATION:_____

COLLECTION, PRESERVATION, AND OTHER SOPs USED:_____

WERE SAMPLES STORED BEFORE TRANSMISSION?_____

IF SO, STORAGE TIME, METHOD, AND LOCATION:_____

_____

MODE OF SAMPLE DELIVERY (HOW ARE YOU SENDING THE SAMPLES?):_____

_____

ANALYSES REQUESTED:_____

_____

DESCRIBE ANY DOCUMENTS ACCOMPANYING THE SAMPLES (CHAIN OF CUSTODY FORM IS REQUIRED):_____

_____

CERC - WORKSHT.II - Rev. Date: March 24, 2016

SAMPLE COLLECTION & SHIPMENT RECORD (continued)

ADDITIONAL NOTES AND COMMENTS:_____

_____

_____

_____

_____

Please ship to:

Jesse Arms
USGS-CERC
4200 New Haven Rd.
Columbia, MO 65201
jarms@usgs.gov
(573)876-1856

CONTACT INFORMATION FOR INDIVIDUAL RESPONSIBLE FOR THE SAMPLES:

NAME:_____

ADDRESS:_____

_____

_____

PHONE:_____

FAX:_____

E-MAIL:_____

A9-2 Field Data sheet -Mussel data

fill in blocked site info on all pages

Ecological Specialists, Inc.

Page _____ of _____

Project no._____ Date_____/_____/_____ Collectors:_____

Site Subsite Circle IDer and square diver

Collection Type: Dive / Bank Search / Wade / Snorkel
Collection Method: Qual. / Quan. / Semi-Quan / Recon.
Sub-Method: Timed Search / Quadrat / Transect / Relocation
Effort min m^2 m

State_____ County_____ River_____ Habitat_____
Bank_____ Pool_____ RM UP_____ DN_____ (e.g., Pool, Riffle, Run, Channel Border, Main Channel, Secondary Channel)
Comments

	Subsite	Rep-licate	Distance from bank min / max	Depth min / max ft or m	Substrate (one per sample) Be	Bo	Cb	Gr	Sd	St	Cl			Species	# Indiv. (L / FD / WD / SF)	Age (annuli ct. or A / J)	Length (mm or ≤ 30 mm)	1 Repro-ductive Condition	2 Zebras / Unionid	3 Zebras / 1/4 m^2	4 % In-fested	Notes/GPS/ Depthfinder/Pic Info./if marked, mussel ID
1																						
2																						
3																						
4																						
5																						
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30																						

1 = reprod cond : M=male, FG=F charging, FG=F gravid, FNG=F not gravid
2 = actual no. zebras or 0, 1-10, 11-50, >50
3 = total no. zebras per 0.25m^2 quadrat = loose zebras + all zebras on unionids
4 = % of shell covered; 0, 1-10%, 1-50%, 51-100%

Environmental conditions

Gage used:_____
River stage (ft):_____
River discharge (cfs):_____
Weather:_____
Air temp:_____ °C °F
Secchi disk depth (cm):_____

Current velocity (surface):_____ ft/sec m/sec
Current velocity (bottom):_____
Water temp (surface):_____ °C °F
Water temp (bottom):_____ °C °F
DO (surface):_____
DO (Bottom):_____

pH (surface):_____
pH (bottom):_____
Cond (surface):_____
Cond (bottom):_____
Redox (surface):_____
Redox (bottom):_____

Data summary

No. species:_____
No. unionids:_____
CPUE:_____
Density:_____
T&Es:_____

A9-3. Example of field data sheet for sediment collection

1. Area of Interest and Water Body: _____

2. CERC Sample ID#: [ ] 2006 Station ID#: _____

3. Names of personnel: _____

4. Alternate station accessed: [ ] Yes [ ] No
   If yes, Alternate ID Code (from Table 3 of SOP): _____
   Reason for Alternate Station Accessed: _____

5. Sampling Date: _____ Time: _____

6. Sampling method: [ ] Boat [ ] Wading

7. Target Sample Coordinates (UTM): Easting _____ Northing _____
   Actual Sample Coordinates (UTM): Easting _____ Northing _____
   Datum: UTM NAD 83 Zone 15N
   Instrumentation: _____
   Problems Encountered: _____

8. Type of water body: [ ] River [ ] Stream [ ] Intermittment tributary
   [ ] Lake [ ] Other _____

9. Sample type (e.g., Moderate Risk): _____

10. Type of sampler used: [ ] Ekman Dredge [ ] PVC Sediment Scoop [ ] Ponar Sampler

11. Sample depth: Target 0 - 8 cm Actual [ ] Same Other: _____

12. Sample volume collected (target): 20 L sediment; 15 L overlying water
    Sample volume collected (actual): _____

13. Sediment type: [ ] Bedrock [ ] Boulders [ ] Cobbles
    [ ] Gravel (0.1 - 2.5") [ ] Sand (<0.1") [ ] Silt [ ] Clay (slick)
    [ ] Detritus [ ] Other _____

14. Sediment Color (black, brown, grey, etc.): _____

15. Sample odor (if readily apparent; e.g., sulfur, oily, sewage, none, etc; do not intentionally smell sample to evaluate odor): _____

16. Water depth : Average _____ Range _____

17. Additional Comments: _____

Appendix 10 Chain of Custody

A10-1 Chain of Custody– general

U.S.Department of the Interior
U.S.GEOLOGICAL SURVEY
Columbia Environmental Research Center
4200 New Haven Road
Columbia, MO 65201

Chain-of-Custody Record

Study No.	Study Name	
Samplers: (Printed)		page___ of ___

Sample Identification	Date	Time	Type *	Remarks and Observations

Relinquished by: (Signature)	Date/Time	Received by: (Signature)	Relinquished by: (Signature)	Date/Time	Received by: (Signature)

Relinquished by: (Signature)	Date/Time	Received for Laboratory by:(Signature)	Date/Time	Remarks

* W=water, S=sediment, P=plant, F=fish, B=benthos, O=other, define in remarks

CERC Form# 32-005 - CHAINOC.WPD - Rev. Date: March 19, 2009

A10-2. Example of chain of custody data sheet – sediment.

Project Name:	2007 TSMD Sediment Sampling Program		

Sample ID#	Sample Type	Sample Description/Comments	
Transfer of Samples from Sampling Team to Sample Runner			
Relinquished By:			
Print Name	**Signature**	**Date**	**Time**
Received By:			
Print Name	**Signature**	**Date**	**Time**
Transfer of Samples from Sample Runner to Truck Inventory Team			
Relinquished By:			
Print Name	**Signature**	**Date**	**Time**
Received By:			
Print Name	**Signature**	**Date**	**Time**
Transfer of Samples from Truck Inventory Team to CERC Laboratory			
Relinquished By:			
Print Name	**Signature**	**Date**	**Time**
Received By:			
Print Name	**Signature**	**Date**	**Time**

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Appendix 11 – Photograph documentation protocol

This guidance describes procedures for the collection and handling of digital photographs taken during natural resource damage assessment field studies. It is intended to protect the legal integrity of digital photographs stored on digital memory cards (e.g., SD cards), as well as the legal integrity of the memory card itself.
Camera memory cards will be issued to field members in order to record photographic images in the field and transfer those images to the Tri-State Mining District (TSMD) mussel study Data Quality Officer.

There are two concepts that apply to creating a legally defensible photo record.

- Maintain a complete photo record. Do not delete photos from the camera or from your computer before the official archive is created.
- Keep one set of photos that are never opened ("archive" set). In practice, this means transferring one copy of the photos from the SD card to a computer, to other storage media such as a non-editable DVD-R or CD-R, or to a secure server, without ever opening the photos. The resulting continuous set of photo files that have not been opened will demonstrate that that you have a full, un-edited, photo record for the court. "Un-edited" also means that the photos' electronic file names generated by the camera are NEVER changed.

Tracking Memory Cards
Each memory card receives a unique ID Number, and its use is tracked through the field work documentation and *Chain of Custody* records. The unique Memory Card ID Number (e.g.TSMD - siteX) is assigned by the FWS Office and legibly printed on the memory card itself with a permanent marker.

At the time of issue, the TSMD mussel study Data Quality Officer representative will record the details of each camera memory card on the appropriate *SD Digital Memory Card Tracking Log* (see Figure 1). This log will be the responsibility of the TSMD mussel study Data Quality Officer. Tracking logs should be kept in the Data Quality Officer's station.

The individual memory card will then be issued to a field team member using a *Chain of Custody* form

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Figure 1: Example of a *Tracking Log* showing two SD cards issued to field team personnel.

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SD Digital Memory Card Tracking Log
Wildlife Operations - Mobile

Date	SD Card ID#	Brand	Memory Capacity (GB)	Issued By	Issued To	Returned? (Y/N & Date)
06Jul10	WO- 1	Kingston	2 GB	John Doe	Bill Smith	
07Jul10	WO- 2	SanDisc	1 GB	John Doe	Fred Hoodie	
	WO- 3					
	WO- 4					
	WO- 5					
	WO- 6					
	WO- 7					

Figure 2: Example of *Chain of Custody* form for the first SD card used by field team.

US Department of the Interior
U.S. Fish and Wildlife Service
Division of Law Enforcement

CHAIN OF CUSTODY RECORD

FILE NO.
INV.

DATE AND TIME OF SEIZURE: N/A

REGION: 3

EVIDENCE/PROPERTY SEIZED BY: N/A

SOURCE OF EVIDENCE/PROPERTY (person and/or location):
☐ TAKEN FROM:
☒ RECEIVED FROM: John Doe
Team 1, TSMD mussel study
☐ FOUND AT: Joplin, MO

CASE TITLE AND REMARKS:
USGS - CERC
4200 New Haven Road
Columbia, MO 65201
Attn: Chris Ivey

ITEM NO. 1

DECRIPTION OF EVIDENCE/PROPERTY (include Seizure Tag Numbers and any serial numbers):

SD Digital Memory Card

Brand or Manufacturer: Cannon

Card Serial # or ID WO-1

Capacity (in GB): 2 GB

Field Team ID: Team 1

State/Operational Area: TSMD - Sites 1-10
(ACP Grid or geographic location)

Date Range Memory Card was in Use: **Installed:** ____ **Removed:** ____

ITEM NO.	FROM: (PRINT NAME, AGENCY)	RELEASE SIGNATURE:	RELEASE DATE:	DELIVERED VIA:
1	John Doe	John Doe	06Jul10	☐ U.S. MAIL
	TO: (PRINT NAME, AGENCY) Bill Smith	RECEIPT SIGNATURE: Bill Smith	RECEIPT DATE: 06Jul10	☒ IN PERSON ☐ OTHER:

Chain of Custody

The person named on the *Chain of Custody* record for a memory card is the person ultimately responsible for the security of that card at all times, until custody of the card is transferred to another person. All such transfers will be documented on the *Chain of Custody* record.

Each memory card will be traced by its own *Chain of Custody* form. So that the labeled SD card and *Chain of Custody* form do not become separated, place them together in a 1-gallon ziplock-type bag. *Ensure only one memory card and one Chain of Custody form per bag.*

At the time the memory card is installed in the camera, the field team member will record the date on the *Chain of Custody* form in the space labeled "Installed". The *Chain of Custody* form should then be placed back in the ziplock-type bag and safeguarded by the field team for later use.

Taking Photographs in the Field

When a photograph is taken during a field study, field teams will:

- Before beginning the field work, record the SD Card ID Number (e.g., "TSMD siteX") in the appropriate space on the datasheet. The camera ID number may also be recorded.
- Record the Photo ID Number (from the camera) in the designated space on the datasheet.

Never erase or over-write any photos or other data existing on the memory card, even if a photo may have been taken by mistake. Record the "mistake" and that photo's ID Number in the Comments section or other area of field study datasheet. Never renumber or change the electronic name of a photograph in the camera or on the memory card.

Archiving Before Viewing Photographs

The legal integrity of the photographs relies upon them NEVER being viewed or opened or altered in any way. At the end of each field day, the photographs should be copied "as-is" from the memory card to a computer, to other storage media such as a non-editable DVD-R or CD-R, or to a secure server if provided by the Trustees, without ever opening the photos. This will be the "Archive Set" of photos and will NEVER be viewed/opened. A second set of photos can be copied to a computer and labeled "Working Set"—only these photos can be viewed/opened, if needed. Field teams should strive to generate an Archive Set at the end of each field day, or as frequently as feasible, in order to create a back-up in case the camera or memory card accidentally becomes damaged.

Taking Memory Cards Out of the Camera

The memory card should remain in the camera until it approaches its storage capacity. Field Team members must transfer custody of the memory card by completing the appropriate

section of the *Chain of Custody* form. (Note, a separate *Chain of Custody* form for the transfer of the camera itself may be necessary, as well.) When it is time to replace the memory card, the field team member in custody of the card will remove it from the camera and move the locking tab to the "Locked" position. On the *Chain of Custody* form, the date will be recorded in the space labeled "Removed". The memory card and *Chain of Custody* form should then be placed back in the ziplock-type bag for return to the TSMD mussel study Data Quality Officer.

If the card is sent via FedEx, ensure that the appropriate "From" box on the *Chain of Custody* form is completed; the TSMD mussel study Data Quality Officer will complete the "To" box upon receipt. Contact the Chris Ivey TSMD mussel study Data Quality Officer at 573-441-2962 for a FedEx account number to which to charge the shipping. Memory cards should be shipped to the following address:

U.S.G.S. Columbia Environmental Research Center
4200 New Haven Road
Columbia, MO 65201
ATTN: Chris Ivey

Important reminders:

- ***Maintain Chain of Custody.***
- ***Record photo ID Numbers on the datasheet.***
- ***At no time will information stored on a digital memory card or camera internal memory be erased or over-written.***
- ***Digital photo files must be stored sequentially on the SD card and not renamed.***
- ***NEVER view or open the photos in the "Archive Set." Only view/open the photos from the "Working Set."***

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