DRAFT ADDENDUM DAMAGE ASSESSMENT PLAN FOR SOUTHEAST MISSOURI LEAD MINING DISTRICT: MADISON COUNTY MINES SITE

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Missouri Department of Natural Resources

U.S. Fish and Wildlife Service
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EXECUTIVE SUMMARY

Mining and smelting sites within Madison County in Missouri are located within the Southeast Missouri Lead Mining District (SEMOLMD), an area that was mined extensively for lead and zinc for more than a century. As a result of this mining and related activities, large amounts of metals including cadmium, lead, zinc, and nickel were released and are continuing to be released into Missouri's environment. Cadmium, lead, zinc, and other metals associated with mining are potentially toxic to a wide variety of plants and animals.

Under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), and implementing regulations, the Director of the Department of Natural Resources (MDNR) and the U.S. Department of the Interior (DOI) are Trustees for natural resources in the Madison County Mines Site (MCM). 40 C.F.R. § 300.600 and § 300.605. Natural resources over which MDNR exercises trusteeship include surface waters (rivers, lakes, streams, etc.), ground water, soils, air, plants, and animals; DOI has trusteeship for natural resources managed or controlled by the DOI, including migratory birds and endangered species, and their supporting ecosystems. As Trustees, the State of Missouri and DOI act on behalf of the public for these resources affected by the MCM Site and have the authority to assess whether the resources and their services have been injured as a result of release(s) of hazardous substances. (See generally 43 C.F.R Part 11) Injuries to natural resources can occur if the resources are exposed for some time period to concentrations of hazardous substances that are high enough to cause specific adverse effects. For example, injuries can occur if lead and/or zinc concentrations in surface waters are so high that relevant water quality criteria are exceeded. Plants and animals are injured if they die, cannot reproduce normally, become sick or are otherwise negatively affected as defined under relevant laws and regulations. 43 C.F.R. § 11.62.

If the Trustees determine that release(s) of hazardous substances have injured natural resources, the Trustees may pursue compensation (damages) to restore, rehabilitate, replace, or acquire the equivalent of the injured natural resources and their services. The Trustees collect compensation from the party or parties determined to be legally responsible for the releases, referred to as potentially responsible parties (PRPs). The Trustees then use the compensation (typically money) recovered to restore, rehabilitate, replace or acquire the equivalent of the injured natural resources. 42 U.S.C. § 9611(i).

The processes through which the Trustees evaluate injuries to natural resources associated with the release(s) of hazardous substances, determine appropriate compensation for those injuries and plan and implement restoration is called natural resource damage assessment and restoration (NRDAR). DOI promulgated and published NRDAR regulations which implement the natural resource damages provisions in the CERCLA. 43 C.F.R. Part 11. These CERCLA NRDAR regulations provide procedures by which trustees can identify natural resource injuries, quantify those injuries, determine appropriate damages for the injured resources and the services they provide, and restore those injured resources. The NRDAR process includes a number of different phases, specifically:

- Pre-assessment
- Assessment planning

- Assessment
- Post-Assessment Planning and Implementation

The Fish and Wildlife Service (FWS), on behalf of DOI, and the State of Missouri have been pursuing NRDAR for portions of the SEMOLMD: Big River Mine Tailings Site and the Viburnum Trend. In July 2014, the Trustees completed the Pre-Assessment Phase for the Madison County Mines Site, which culminated in a Pre-Assessment Screen (PAS) and Determination (MDNR and DOI 2014). In the PAS, in accordance with applicable regulations, the Trustees concluded that further investigation and assessment of natural resource injuries and damages were warranted at the Madison County Mines Site. (43 C.F.R §§11.24 and 11.25).

The Trustees are in the assessment planning phase of NRDAR. (43 C.F.R §§ 11.30 – 11.38). The purpose of an Assessment Plan "is to ensure that the [natural resource damages] assessment is performed in a planned and systematic manner and that methodologies selected for the Injury Determination, Quantification, and Damage Determination phases, can be conducted at a reasonable cost." (43 C.F.R. § 11.30). In 2009, the Trustees released a Phase I Natural Resource Damage Assessment Plan (DAP) for a portion of the SEMOLMD that included the Big River Mine Tailings Site and the Viburnum Trend[1].

This document will serve as an addendum to the earlier DAP and will serve to incorporate the Madison County Mines (MCM) Site into the SEMOLMD DAP. The Addendum describes the affected natural resources in the MCM Site and the activities and studies that the Trustees currently intend to pursue in the MCM Site assessment (see Exhibit ES-1).

Both the Addendum and the DAP are living documents and continually will be developed and refined as the NRDAR progresses and additional information becomes available.[2] Potential changes to this Addendum may include the addition of new studies and/or the modification of the planned studies identified in this document as well as other documents prepared by the Trustees during the NRDAR process, such as a Restoration Compensation and Determination Plan.

At this time, the Trustees' assessment activities are focused on four resources: surface water, geological, groundwater, and biotic resources. Assessment activities will include comparing site specific data collected from previous investigations to literature benchmarks such as aquatic life criteria, sediment quality guidelines, ecological toxicological benchmarks for soil, maximum contaminant levels for drinking water, in order to determine if there are exceedances constituting injury. In addition, the Trustees plan to conduct site specific studies. Specific studies anticipated to date include transition zone soil characterization, songbird exposure investigations, and crayfish population and in-situ toxicity studies. A draft of the crayfish population and in-situ

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[1]See http://www.fws.gov/midwest/es/ec/nrda/SEMONRDA/documents/finalapsemomdphase11-7-09.pdf for Final Phase I Damage Assessment Plan for Southeast Missouri Lead Mining District: Big River Mine Tailings Superfund Site, St. Francois County and Viburnum Trend Sites, Reynolds, Crawford, Washington, and Iron Counties.

[2]The CERCLA NRDAR regulations allow an Assessment Plan to "be modified at any stage of the assessment as new information becomes available." 43 CFR §11.32(e).

toxicity study plan is appended to this Addendum. Draft of plans for other studies will be made available to the public for review and comment as they are developed.

The opinions, suggestions, and other input of the public are important factors that the Trustees consider when making decisions during the course of a NRDAR. A number of documents produced during the course of the NRDAR will be released to the public for review and comment. Specific anticipated opportunities for public involvement include commenting on this Addendum, assessment work plans, and any restoration plans. Each public comment period will last for at least 30 days. Comments may be submitted in writing to one or both of the addresses below.

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The Trustees also recognize the special interests of Madison County landowners. Much of the land is privately held and conducting some of the assessment activities described in this Addendum will require access to private properties. The Trustees understand that this access is dependent upon the permission of the landowners and will work with landowners to secure the needed permissions before beginning any fieldwork activities.

The complete list of companies that mined at the MCM Site over the years is extensive; some no longer exist due to bankruptcy, dissolution, buyouts, mergers or similar corporate events. A list of companies that engaged in mining and/or mining-related activities at OU-2 releasing hazardous substances into the MCM environment, or activities that exacerbated the situation include: Anschutz Mining Corporation and NL Industries Inc. The Trustees may identify other PRPs in the course of pursuing this NRDAR.

In 2009 the Trustees received compensation for injuries to natural resources and the services they provide at the Little St. Francis River Chat Pile and Catherine Mine under the ASARCO Bankruptcy settlement.

In accordance with the CERCLA NRDAR regulations, in July 2013, the Trustees sent the identified PRPs a Notice of the Intent to Perform an Assessment, along with a copy of the a PAS,

and invited them to participate in a cooperative assessment (43 C.F.R. § 11.32(a)(2)(iii)(A)). If the Trustees reach an agreement with any or all of the identified PRPs to conduct a cooperative assessment, the Addendum will be updated to reflect that relationship and describe any updates or modification in assessment activities. The revised Addendum will be made available for public comment and review. As the MCM Site NRDAR progresses, the Trustees retain the right to participate in cooperative assessment activities with one or more PRPs at any time.

Exhibit ES-1: Assessment Activities for Madison County Mines Site NRDAR

NATURAL RESOURCE(S)	STUDY	STATUS
INJURY DETERMINATION AND QUANTIFICATION		
Terrestrial Habitat and Geologic Resources	Soil metal characterization in transition zones near mine and mill sites	Potential
Aquatic Biota	Crayfish population and toxicity	Planned
Terrestrial Biota	Small Mammals	Potential
Terrestrial Biota	Songbird Exposure	Potential
Ground Water	Exceedances of Regulatory Standards	Potential
Geologic Resources	Exceedances of Literature-Based Impact Thresholds	Planned
PATHWAY DETERMINATION		
All	Fate and Transport	Planned
DAMAGES DETERMINATION		
Aquatic Habitat	Primary Restoration Estimate	Post injury studies
Terrestrial Habitat	Primary Restoration Estimate	Post injury studies
Terrestrial Habitat	Habitat Equivalency Analysis	Post injury studies
Ground Water	Replacement Cost Estimation	Potential

The public can learn more about the MCM Site NRDAR by visiting the website:
http://www.dnr.mo.gov/env/hwp/sfund/nrda.htm
and/or
http://www.fws.gov/midwest/semonrda

CHAPTER 1 INTRODUCTION

The Madison County Mines Site is a subdistrict of the Southeast Missouri Lead Mining District (SEMOLMD). The SEMOLMD is a large area of historic and current lead and other heavy metals mining that is comprised of two main subdistricts: the Old Lead Belt and the Viburnum Trend (also known as the New Lead Belt). (See Exhibit 1 for a map of SEMOLMD.) The Phase I Natural Resource Damage Assessment Plan (DAP) described proposed natural resource damage assessment activities within the SEMOLMD at the Big River Mine Tailings Site and the Viburnum Trend. This document will serve as an addendum to the DAP and will incorporate the Madison County Mines Site into the assessment activities underway in the greater SEMOLMD natural resource damage assessment and restoration (NRDAR). The Madison County Mines (MCM) Site is described in more detail in Sections 1.1 and 1.2.

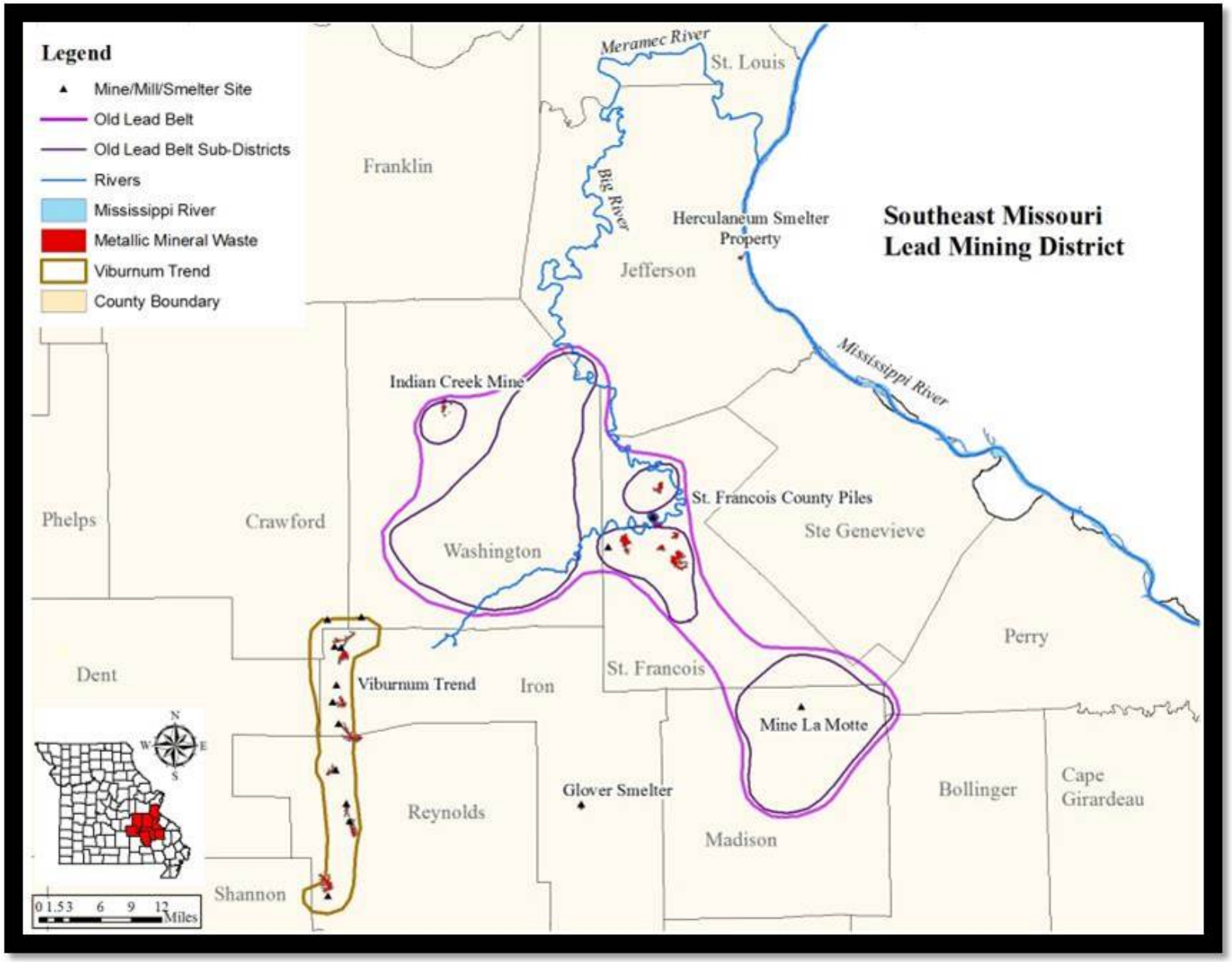
The Old Lead Belt is located approximately 60 miles south – southwest of St. Louis and includes, in addition to St. Francois County, parts of Jefferson, Franklin, Washington, Madison, Perry, and St. Genevieve Counties. The Madison County mining area is sometimes referred to as a separate sub-district from the rest of the Old Lead Belt in St. Francois County due to some distinctions in mineralogy and geology. For purposes of this NRDAR document, we have included Madison County into the Old Lead Belt. The Old Lead Belt lies on the eastern edge of the Ozark Uplift, characterized by rolling hills dissected by narrow floodplain, creek, and river valleys.

NRDAR, as set forth in the CERCLA NRDAR regulations, (43 C.F.R. Part 11) is designed to be an iterative process, meaning data is collected and evaluated and the plans for assessment activities can be added to or revised over time. Likewise, an assessment plan is a living document, and as previously mentioned, may be modified or amended as the extent of injury is evaluated and new NRDAR needs identified. See the Final Phase I Damage Assessment Plan for Southeast Missouri Lead Mining District: Big River Mine Tailings Superfund Site, St. Francois County and Viburnum Trend Sites, Reynolds, Crawford, Washington, and Iron Counties (Mosby et al. 2009) which provides an overview of the NRDAR process. (http://www.fws.gov/midwest/es/ec/nrda/SEMONRDA/documents/finalapsemomdphase11-7-09.pdf)

1.1 MADISON COUNTY MINES SITE DESCRIPTION

The Madison County Mines Superfund Site is located near Fredericktown, Missouri at the southern end of the Old Lead Belt Mining District of Southeast Missouri on the southeastern edge of the Ozark Uplift in Missouri. The principal drainage system for the Madison County Mines Site is the south flowing Little St. Francis River and its tributaries.

Exhibit 1: Map of the Southeast Missouri Lead Mining District



The Madison County Mines Superfund Site contains six operable units (See Exhibit 2).

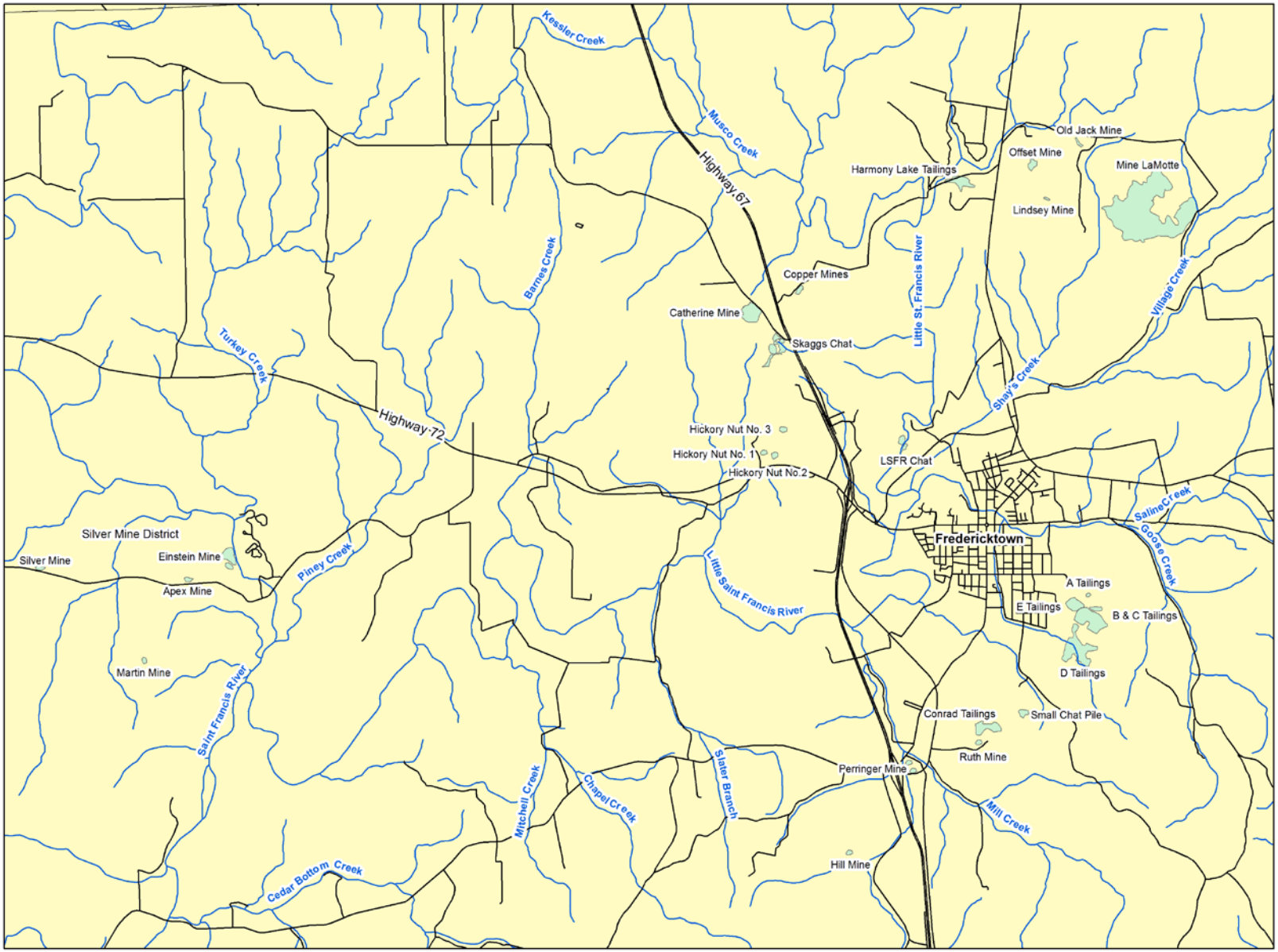
- OU1 – Northern Madison County Unit, including Mine La Motte Recreational Area (MLMRA), Harmony Lake, Copper Mines (also known as Basler Mines), Lindsey Mine, Offset Mine, and Old Jack Mine
- OU2 – Anschutz Subsite, including A, B, C, D, and E Tailings Areas (historically known as Madison Mine)
- OU3 – Madison-Wide Residential
- OU4 – Conrad Tailings and Ruth Mine
- OU5 – Catherine Mines, Skaggs Piles, the Little St. Francois River Subsite (LSFR), and the transect of the overhead tram from the Skaggs subsite to the LSFR subsite.
- OU6 – Hickory Nut Mines and Silver Mines
- OU7 – Little St. Francis River Watershed

The MCM PAS addressed three of the major mine, mill, and/or smelter sites in the Madison County Mines Superfund Site where potentially responsible parties (PRPs) may have NRDAR liability. The Trustees will be focusing assessment efforts on Operable Unit 2, which includes the Anschutz Subsite for NRDAR. Streams draining OU2 include Little St. Francis River, Spiva Branch, ,Goose Creek, Tollar Branch, Saline Creek, and possibly other named and unnamed tributaries to the Little St. Francis River. Please see Appendix A for a map of OU2.

1.1.1 Response Activities at the MCM Superfund Site

The MCM was placed on the National Priorities List under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) in 2003. A variety of actions have been taken at the MCM Superfund Site by the U.S. Environmental Protection Agency (EPA); some under agreements with PRPs, some under remedial authorities pursuant to Records of Decision, and some as fund-lead under EPA's removal authority. The removal actions have focused on excavating contaminated residential yards and supplying bottled water to residents with contaminated wells. Hundreds of residential yards are potentially contaminated from mill waste transport and erosion within the MCM Superfund Site. The majority of the Anschutz Tailings (historically known as Madison Mine) property in OU2 was stabilized and capped under an agreement with EPA. Records of Decision have been filed for OU3 (residential properties, which included the Little St. Francis River subsite), OU4 (Conrad Tailings), and OU5 (Catherine/Skaggs Piles).

Exhibit 2: Map of Madison County Mines Superfund Site



LEGEND

- Streams
- Roads
- Madison County Piles

0 0.5 1 2 Miles

N

Map created by Kathy Rangen April 2015.
This map can be found at M:/Superfund/NRD Southeast_Miningi/Madison Co Sediments/MadCoForDAP..mxd.
Although all data sets used to create this map have been compiled by the Missouri Department of Natural Resources, no warranty, expressed or implied, is made by the department as to theaccuracy of the data and related materials. The act of distribution shall not constitute any such warranty, and no responsibility is assumed by the department in the use of these data or related materials.

Missouri Department of Natural Resources
Division of Environmental Quality
Hazardous Waste Program

Exhibit 3: Tailings and Chat Distribution in the MCM Site

Site	Estimated Tailings/Chat Acreage	Estimated Transition Zone Acreage	Total Acreage
OU2 (Madison Mine) (Total	146	118	265
- A Tailings	3	8	11
- B Tailings	4	9	13
- C Tailings	44	28	72
- D Tailings	70	49	119
- E Tailings	18	15	33
- Small Chat Pile	7	9	16

* Mine wastes no longer visible from aerial photography.

There is also an area-wide remedial investigation (RI) that characterizes the nature and extent of contamination on and away from the piles that was written in 2008. As part of that RI, an Ecological Risk Assessment (ERA) for OU3 was completed by the EPA in 2006. Since then a Supplemental Remedial Investigation/Focused Feasibility Study (RI/FS) was initiated in 2011 for OUs 1, 2, 3, 5 and 6. The RI/FS for OUs 1, 2 and 6 remain in draft form while the others have been completed. This includes the supplemental ERA for OUs 1, 2 and 6.

The supplemental ERA found adverse impacts to terrestrial and aquatic habitat from mining activities throughout the MCM Site. The ERA indicated that the MLMRA Slime Pond, the wetland at Copper Mines and the Met Pond on the Anschutz Site all pose an acute and chronic toxicity risk to benthos. There is also clear evidence of phytotoxicity to terrestrial plants in mine waste areas. Hazard quotient (HQ) analyses of herbivores (>100) show a high likelihood of toxic effects from mining activities at the Old Jack Mine and Anschutz Site. Terrestrial vermivores show the same risk (HQ >100) for metals toxicity at the Copper Mines Tailings Area and the Old Jack Mine.

Exhibit 4: Chronology of CERCLA Activities at the MCM Site Compiled by EPA in a 2013- 5 Year Review.

Event or Activity	Date
Missouri Department of Conservation Investigation of Flood Event	1980
MDNR Preliminary Assessment of Presence of Hazardous Waste	July 1983
MDNR Preliminary Assessment/Site Investigation	February 1986
Site Characterization Report	1990
Final Expanded Site Inspection Report	July 1995
Administrative Order on Consent for Site Characterization	March 1999
Final Report on PRP Search	January 2000
Administrative Order on Consent for Harmony Lake Removal Action	June 2000
Removal Action on Harmony Lake	September 2000
Administrative Order on Consent for Removal Action at Harmony Lake	November 2000

PRP Characterization Summary-Draft Report	November 2000
Madison County Health Department Request for EPA Assistance	June 2002
Unilateral Administrative Order on Consent for Removal Response Activities	August 2002
Removal Action-Fredericktown Farm Supply	August 2002
Removal Assessment-Madison Mine/Fredericktown	August 2002
Removal Action- Madison Mine/Fredericktown	March 2003
Administrative Order on Consent for Removal Site Investigation	June 2003
Site Listing to the National Priority List	September 2003
Removal Assessment Summary Report	February 2003
Removal Action Madison County Mines	August 2004
ASTDR Public Health Assessment	April 2005
Feasibility Study	July 2005
Ecological Risk Assessment	May 2006
Removal Action Summary-Madison County Mines	April 2007
Final Baseline Human Health Risk Assessment	July 2007
Final Area-Wide Remedial Investigation Report	April 2008
Removal Action Summary Report-Fredericktown	June 2008
Interim Record of Decision OU3-Residential Soils	February 2009
RA Start OU1-Resdential Soils	February 2009
Feasibility Study OU4-Conrad Tailings	May 2011
Record of Decision OU4-Conrad Tailings	September 2011
Feasibility Study OU5-Catherine Mine and Skaggs Tailings	June 2012
Record of Decision OU5- Catherine Mine and Skaggs Tailings	September 2012
Remedial Design-OU4-Conrad Tailings	September 2012

1.2 NATURAL RESOURCE DAMAGE ASSESSMENT ACTIVITIES AT MCM SITE

The Trustees have completed the Pre-Assessment Phase for the MCM Site NRDAR. The main product of the pre-assessment phase is the MCM Site PAS, which was issued in June 2014.[3] In the PAS, the Trustees confirmed the following:

(a) Heavy metals have been and are being released into the environment;
(b) Natural resources have been adversely affected by these releases;
(c) Contaminant concentrations are sufficient to injure natural resources;
(d) The data needed to conduct NRDAR are available or can be obtained at a reasonable cost; and
(e) Completed or planned response actions would neither completely restore the injured natural resources nor compensate the public for the injuries.

[3] The PAS is available at: http://www.dnr.mo.gov/env/hwp/sfund/nrda.html and http://www.fws.gov/midwest/semonrda

Based on these criteria, the Trustees decided to pursue additional NRDAR activities. To that end, the Trustees developed this draft Addendum to the DAP as the assessment plan for the MCM Site. This Addendum describes activities that will collect and generate information for determining the nature and extent of natural resources injuries and contaminant pathways.

This Addendum summarizes Chapter 2 (Background) from the Assessment Plan. More specifically this Addendum discusses the MCM Site's natural resources; identifies the primary hazardous substances of concern; and describes the processes that resulted in the releases of the hazardous substances to the sites and surrounding areas. The remaining portions of the Assessment Plan, as listed/described below are not covered in this document to prevent unnecessary duplication. The information in those chapters can be found in the original Assessment Plan, which can be found at the link given in footnote 2.

- Chapter 3 (Role of Trustees) identifies the Trustees, describes the nature of their trusteeship, and provides an overview of the NRDAR process that the Trustees plan to follow.
- Chapter 4 (The Southeast Missouri Lead Mining District NRDAR) provides an overview of studies proposed at the time the DAP was written (2009). This Addendum provides an overview of proposed or contemplated studies for MCM Site only.
- Chapter 5 (Quality Assurance Management) establishes the general procedures used in developing project-specific quality assurance plans.

CHAPTER 2 AFFECTED NATURAL RESOURCES AT AND FROM THE MADISON COUNTY MINES SITE

The MCM Site supports a variety of natural resources potentially affected by mining-related contamination, including rivers and lakes, ground water, terrestrial and aquatic organisms, and geologic/terrestrial resources. As described in the Final Pre-Assessment Screen and Determination for Madison County Mines Site, Madison County, Missouri (USFWS and MDNR 2014), these habitats support a wide variety of fish, birds, and other wildlife. A number of species present in the area are included on state or federal threatened and endangered species lists or are otherwise of special concern. The following paragraphs briefly summarize key features of the assessment area's natural resources, including what makes the area unique and the threat posed to these resources by mining-related contamination.

2.1 SURFACE WATER RESOURCES: RIVERS AND STREAMS

Surface water resources are defined as "the waters of the United States, including the sediments suspended in water or lying on the bank, bed, or shoreline" (43 C.F.R. §11.14(pp)). Exhibits 8 through 12 (in the SEMOLMD DAP) and the following paragraphs summarize key information about the area's surface water resources, including a brief description of each river or creek, biota supported by each waterway, and potential contamination.

The MCM contains numerous Ozark streams, all tributaries to the St. Francis River. Ozark-type streams are typically characterized as clear, with good water quality, high hardness, moderate gradient, gravel and bed-rock dominated, with riffle-pool complexes. Higher order Ozark streams are typically spring-fed and are dominated by groundwater recharge at low flow.

2.1.1 St. Francis River and Tributaries Surface Water

As discussed in the PAS (USFWS and MDNR 2014), the principal drainage system for MCM Site is the Little St. Francis River and its tributaries. EPA conducted surface water sampling in the Little St. Francis River and its tributaries in 2006. The National Ambient Water Quality Criteria (NAWQC) for Cd, Copper (Cu), Ni, Pb and Zn was calculated using site specific hardness data collected from the water sample. Maximum concentrations of dissolved Pb (21.5 µg/l) in Sweetwater Branch exceeded the chronic NAWQC for Pb (4 µg/l). Dissolved Cu (25.3 µg/l) from Spiva Branch exceeded the chronic NAWQC for Cu (7 µg/l) and the chronic NAWQC for Ni (113 µg/l) was also exceeded with dissolved Ni (3480 µg/l) from an unnamed creek on the north end of the Anschutz Property.

Additionally, the MDNR listed 1.7 miles of Saline Creek, a tributary of the Little St. Francis River that flows through mine impacted areas, as impaired or potentially impaired in the state's water quality report (Section 305(b) of the Clean Water Act), but it was not approved for the state impaired waters (Section 303(d) of the Clean Water Act), which is approved by the Missouri Clean Water Commission after a total maximum daily load (TMDL) is completed. Saline Creek was listed because of dissolved Ni.

Sediment samples collected in the MCM Site had maximum concentrations of Cd at 34.6 mg/kg, Cu at 10,100 mg/kg, Ni at 5700 mg/kg, Pb at 13,000 mg/kg, and Zn at 2900 mg/kg (Appendix A, Table 3 in SEMOLMD DAP). The highest concentration of Pb was found in sediment in the Little St. Francis River near the Little St. Francis River Chat pile, and the highest concentrations of Cd, Cu and Ni from the Tollar Branch on the Anschutz Property (EPA 2011a).

The 2011 ERA found sediment samples analyzed from tributaries to the Little St. Francis River to have metal concentrations that could adversely impact aquatic life communities. Based on sampling conducted by Environmental Strategies Corporation in 2000, the Little St. Francis River has at least 4 miles of contaminated sediment which exceed the Probable Effect Concentration(PECs) for Pb from the confluence of Logtown Branch to just below the confluence of Sweetwater Branch. A sample taken approximately 0.2 miles below City Lake was also above the PEC for Pb. Many of the tributaries to the Little St. Francis River were also above the PECs for Pb, Cd, Cu, and/or Ni including 1.4 miles of Sweetwater Branch (above and below Mine La Motte Lake), approximately 1 mile of Village Creek, and 6.6 miles of Shays Creek (above and below Slime Pond). MDNR sampling of Saline Creek below the confluence of an un-named tributary that drains the A tailings pond at the Anschutz mine exceeded the PEC for Pb and Ni.

MacDonald et al. (2000) identified consensus-based freshwater sediment quality guidelines: the Probable Effect Concentrations (PECs) and Threshold Effect Concentrations (TECs). PECs represent concentrations of contaminants above which adverse effects on sediment-dwelling organisms are expected to occur frequently. TECs represent concentrations of contaminants in

sediment below which adverse effects on sediment-dwelling organisms are expected to occur infrequently. The TEC and PEC values for cadmium, lead and zinc and copper and nickel appear below in Exhibit 5.

Exhibit 5: TEC and PEC Values for Mining-Related Metals

Contaminant	TEC Value ppm	PEC Value ppm
Cadmium	0.99	4.98
Lead	35.8	128
Zinc	121	459
Copper	31.6	149
Nickel	22.7	48.6

2.2 GEOLOGIC RESOURCES

In the context of a natural resource damage assessment, geologic resources are defined as "those elements of the Earth's crust such as soils, sediments, rocks, and minerals, including petroleum and natural gas, that are not included in the definitions of ground [water] and surface water resources" (43 C.F.R. §11.14(s)).

Exhibit 6: Potentially Impacted Water Bodies

OU	WATERBODY	IMPAIRMENT/REASON
OU1/OU2	Little St. Francois River	Pb/Missouri 303(d); Above PEC for Pb in sediments using Freshwater Sediment Quality Guidelines (MacDonald et al 2000)
OU2 Madison (Anschutz) Mine	Saline Creek	Dissolved Ni/Section 305(b) of Clean Water Act (EPA 2014)
OU2 Madison (Anschutz) Mine	Tollar Branch	Likely heavy metal sediment contamination due to proximity to mining operations and tailings pile.
OU2 Madison (Anschutz) Mine	Goose Creek	Habitat degradation/ Section 305(b) of Clean Water Act
OU2 Madison (Anschutz) Mine	Spiva Branch	Dissolved Cu and Ni/ National Ambient Water Quality Criteria (NAWQC)

In its natural state, the area's soils support diverse ecosystems, such as oak savanna and deciduous woodland. However, many geologic resources within the Missouri SEMOLMD are either currently covered by mine waste piles or fall within the footprints of former piles. Heavy

metals from mine, mill, and smelter wastes can be toxic to soil microbes and reduce the ability of a soil to function in a normal and productive way (Mosby et al. 2009).

Madison County is subdivided into the St. Francois Mountains on the western side and the Salem Plateau on the eastern side of the county. Topographically, the St. Francois Mountains comprise a geologically mature landscape with rounded ridges and meandering streams that occupy comparatively wide valleys. In a few locations, rivers and streams cut across ridges, forming steep canyons (EPA 2014).

Much of the Site is underlain by Paleozoic (Cambrian) sedimentary rocks that rest on Precambrian crystalline rocks or basement complex which form the St. Francois Mountains. The sedimentary formations vary in thickness and locally thin out or pinch out against structural highs of the basement complex (St. Francois Mountains). The rock formations present in the area include the following from the Precambrian basement: (1) the Lamotte Sandstone, (2) the Bonneterre Dolomite, (3) the Davis Formation, and (4) the Derby-Doe Run Dolomite. Soil formed from these formations is predominantly clays with comparatively low permeability. Soil profiles and horizons are generally well developed (EPA 2014).

Most lead mineralization in the Madison County area occurs within the lower part of the Bonneterre Dolomite on the flanks of buried or exposed Precambrian topographic highs, generally within a few hundred feet of the boundary where the underlying Lamotte Sandstone pinches out. Lead ore, primarily in the mineral galena, and other metallic minerals occur as deposits that have replaced dolomite crystals in portions of the Bonneterre Dolomite. The ore occurs in horizontal sheets along bedding planes, cavity fillings and linings on the walls of joints and fractures. The deposits extend laterally for hundreds of feet and may extend 200 feet vertically. However, mineralization in the Silver Mines area is distinct, consisting of quartz veins in the Precambrian basement complex that contain galena, wolframite (iron tungstate) and additional sulfide minerals as primary ore phases for additional metals such as tungsten and silver (EPA 2011).

2.3 GROUND WATER

Groundwater is described as occurring both within unconsolidated overburden soils and bedrock. Groundwater within the overburden materials is less abundant than in the bedrock due to the generally low permeability and thin character of the local soils. Two main aquifers are identified in the area: the Bonneterre Transition Zone and the Davis Formation/Whetstone Creek member (Dames & Moore 1990). These two aquifers are separated by the Lower Bonneterre Formation which serves as an aquitard or confining bed that impedes the exchange of water between the two aquifers.

The Bonneterre Transition Zone is mudstone that grades downward into dolomitic sand. The sand has an estimated hydraulic conductivity on the order of 3.1 feet per day. The Whetstone Creek Member is a medium- to coarse-grained crystalline dolomite with interbedded gray and green shales. This unit is locally a major source of groundwater and is considered to be a more significant water-bearing unit in the area due to its higher hydraulic conductivity estimated at 11 feet per day. Groundwater flow within the region is poorly defined, but under natural or

undisturbed conditions is projected to follow the overall topographic gradients. Flow within both unconsolidated overburden and bedrock is expected to be from upland areas to lower topographic areas such as along the major drainage courses. Mine workings, including open and collapsed stopes, tunnels and rooms, are expected to locally alter groundwater flow. Rates of groundwater flow are unknown but expected to be potentially high based on the aggregate pumping required to dewater the Madison Mine workings, being on the order of 1,000 to 1,500 gallons per minute. Consequently, most of the lead mines within the Bonneterre Formation are expected to be at least partly flooded. Mine workings associated with the Silver Mines area are also expected to be partly flooded based on observations of drainage emanating from some mine adits (EPA 2014).

Groundwater at the Site is predominantly alkaline in nature, attributed mostly to the presence of sedimentary dolomite and limestone. Alkaline groundwater buffers the dissolution of metals and has been attributed as a major reason for the lack of dissolved metals in groundwater outside the former mining and processing locations at the Site where limited detection has been observed. There are an estimated 2000 private wells at the Site potentially used for consumption or, potable water. These include both shallow wells in the unconsolidated overlying soils and deeper wells penetrating the Cambrian sedimentary rock, the Pre-Cambrian basement formations, or both (EPA 2014).

2.4 BIOTIC RESOURCES

2.4.1 Threatened and Endangered Species

Congress delegated responsibility to the FWS for the conservation, including recovery, of federally listed endangered or threatened species, except for marine mammals. The Endangered Species Act and associated federal regulations establish the FWS' authorities for endangered species programs. Several federally threatened and endangered species occur in the MCM Site or near (within Madison County) the MCM Site. The Missouri Department of Conservation's Natural Heritage Database was used to determine threatened, imperiled, or endangered species for this section of the report (http://mdc.mo.gov/your-property/greener-communities/heritage-program; accessed 12/5/2014).

The federally listed endangered gray bat (*Myotis grisescens*) occupies a limited geographic range in limestone karst areas of the southeastern United States. With rare exception, the gray bat roosts in caves year-round. Most gray bats migrate seasonally between hibernating and maternity caves. Gray bats are active at night, foraging for insects over water or along shorelines, and they need a corridor of forest riparian cover between roosting caves and foraging areas. They can travel as much as 20 kilometers (12 miles) from their roost caves to forage. The MCM Site has the potential to impact the gray bat.

The Indiana bat (*Myotis sodalis*), federally listed as endangered, historically occupied much of the eastern half of the United States. The bat hibernates in caves, but during warmer seasons roosts principally under the bark of trees. Maternity colonies are formed mostly in riparian and floodplain forests associated with small to medium-sized streams. They have also been found along tree-lined drainage ditches. Indiana bats are active at night foraging for aquatic insects and

Lepidoptera at a height of 2 to 30 meters over water and under riparian and floodplain trees. The MCM Site has the potential to impact the Indiana bat.

The northern long-eared bat (*Myotis septentrionalis*) is federally proposed as endangered. The range of the bat includes much of the eastern and north central United States. The northern long-eared bat is in danger from extinction due to the spread of white-nose syndrome (WNS). This bat hibernates in caves and mines; during summer the species will roost singly or in colonies underneath bark, in cavities, or in crevices of both live and dead trees. Unfortunately WNS has been found in hibernacula of several counties in Missouri including Iron County which is adjacent to Madison County. The MCM Site has the potential to impact this fragile species.

Habitat requirements for the endangered American burying beetle (*Nicrophorus americanus*), particularly reproductive habitat requirements, are not fully understood at this time. The American burying beetle has been found in various types of habitat, including oak-pine woodlands, open fields, oak-hickory forest, open grasslands, and edge habitat. The MCM Site has the potential to impact the American burying beetle.

The MCM Site has the potential to impact the endangered plant, running buffalo clover (*Trifolium stoloniferum*). Habitat requirements include mesic areas of partial to filtered sunlight where a prolonged pattern of moderate periodic disturbance occurs. It is often found in regions underlain with limestone or other calcareous bedrock. It has been reported in a variety of habitats, including mesic woodlands, savannahs, floodplains, stream banks, sandbars, grazed woodlots, mowed paths, old logging roads, jeep trails, skidder trails, mowed wildlife openings within mature forest, and steep ravines. Mead's milkweed (*Asclepias meadii*) is a federally threatened species that also has the potential to be impacted by mining activities at MCM Site. This milkweed requires moderately wet (mesic) to moderately dry (dry mesic) upland tallgrass prairie or glade/barren habitat characterized by vegetation adapted for drought and fire. It persists in stable late-successional prairie.

Several federally listed freshwater mussel species occur in the vicinity of the MCM Site. Freshwater mussels are bivalved mollusks that are relatively immobile, spending their entire lives partially or completely buried in the stream bottom. They are suspension feeders, using their gills to remove suspended particles in the water column. These animals have a complex life cycle that includes a brief, obligatory parasitic stage on fish. Host fish specificity vary among mussels. While some mussel species appear to require a single host species, others can complete their life cycle on several fish species. The Rabbitsfoot (*Quadrula cylindrical cylindrical*) is a species of mussel that is threatened by the effects from mining at the MCM Site.

The Bachman's sparrow (*Peucaea aestivalis*), a federally endangered species, is a small migratory songbird that breeds in the forests of the central and eastern United States. They nest in areas of scattered scrubby vegetation and build their nests on the ground against or under a grass tuft or low shrub. Nesting areas include dry, open pine or oak woods with brushy or overgrown hillsides or overgrown fields with thickets and brambles. The sparrow forages on the ground for seeds of herbaceous plants or pine and insects. The MCM Site has the potential to impact the Bachman's sparrow.

2.4.2 Vegetation

Prior to settlement, the area had a mixture of forests, glades, open woodlands and small prairies in the basins. Today the more rugged sections of the MCM Site are wooded, and there are extensive acreages of national forest. Large areas of woodlands and igneous glades remain. Open areas and some forested areas are used as cropland, pasture, meadows, and overgrown areas produce grain and seed crops, grasses and legumes, and wild herbaceous plants (Nigh and Schroeder 2002).

Historically, Madison County contained woodlands, forests, glades and cliff communities found on igneous substrates; this community structure was found nowhere else in the Ozarks. Today the area is forested in second-growth oak and oak-pine. Woodlands cover hillsides and riparian corridors. Shortleaf pine was once prevalent but is much reduced in extent (Nigh and Schroeder 2002). The SEMOLMD is the northern extent of short-leafed pine, which was extensively logged in the 19th century. Short-leafed pine still occurs in upland areas of the SEMOLMD including parts of Madison County. Currently, native forests are characterized by a variety of oak species (*Quercus* spp.), hickory species (*Carya* spp.), eastern red cedar (*Juniperus* spp.), dogwoods (*Cornus* spp.) and redbuds (*Cercis* spp.), ash species (*Fraxinus* spp.) and associated shrubs, grasses, legumes, and wild herbaceous plants.

Chat piles and tailings impoundments at the MCM Site do not support normal succession of terrestrial vegetation. Un-remediated mine waste piles tend to be barren or sparsely vegetated. Acres of barren and vegetated mine waste piles have been identified and measured as part of Superfund site investigation activities, and many are readily apparent in aerial photographs.

The loss of vegetation, resulting from phytotoxicity, is an injury under CERCLA NRDAR regulations (43 C.F.R. §11.62(f)(i)). While plant productivity and changes in plant species composition in mine waste areas can be affected by mine waste characteristics unrelated to the presence of contaminants (e.g., the wastes' water retention ability and/or organic content), available evidence suggests that contamination in some areas affected by mine wastes is sufficiently toxic to cause decreases in plant productivity, changes in species diversity, and/or changes in species composition.

Struckhoff et al. (2013) investigated the effects of mining-associated metals (lead and zinc) contamination on native floristic quality. These studies were conducted in the SEMOLMD and used Mean C (Coefficient of Conservatism) and Floristic Quality Index (FQI) as the primary end points for assessing reduction in floristic quality. Lead inhibits the growth of plants, photosynthesis (due to reduced enzyme activity), cell division and respiration, water absorption and other physiological functions of plants (Fargasova 2001). Zinc can cause a decrease in enzymes required for photosynthesis and can decrease hydrolysis in plants that are watered with zinc solutions as low at 650 ug/L (Pahlsson 1989).

Results from the floristic quality study showed that Pb at a concentration in soil of 661 mg/kg can cause a 10% reduction in Mean C and a 10% reduction of FQI at 663 mg/kg. Zinc studies showed that a soil Pb value of 448 mg/kg can reduce Mean C by 10% and FQI can be reduced by 10% at 311 mg/kg of Zn. The research showed that Pb and Zn concentrations in soil and floristic

quality are inversely related. Less tolerant species of disturbance (i.e. have a higher Mean C) are most negatively affected by increasing metals concentrations in the soils. FQI can be very useful for determining community-level effects of soil metals contamination.

2.4.3 Aquatic and Amphibious Species

Aquatic organisms found at the MCM Site include a wide variety of fishes. Among these are a number of larger or recreationally important fish species such as smallmouth bass (*Micropterus dolomieui*), rock bass (*Amploplites rupestris*), longear sunfish (*Lepomis megalotis*), and several sucker species. Smaller fish species include minnows and darters.

Many species of aquatic organisms are present in the MCM Site surface waters, and some may have been and/or continue to be impacted by metals contamination. The longnose darter (*Percina nasuta*) is state listed as endangered and can be found in the St. Francis River watershed. As mentioned in section 2.4.1 above, freshwater mussels occur in the St. Francis River basins. These streams are important refuges for mussel species of concern including the federally endangered rabbitsfoot (*Quadrula cylindrica cylindrica*).

Elevated heavy metal concentrations have been documented in a variety of biological tissues at the MCM Site. Fish, macrobenthic invertebrates, earthworms, plants, and small rodent tissues have been found with elevated metal concentrations. Fish in the Little St. Francis River have shown elevated concentrations of lead and the biochemical effects of lead downstream of the Site (EPA 2006).

EPA Region 7 documented toxicity to *Hyalella azteca* using sediment pore water collected from the Little St. Francis River in the Madison County Mines Site Ecological Risk Assessment (2006). It was further found in the ERA that macroinvertebrate EPT Richness is reduced in the Little St. Francis River as compared to the control site, the Castor River.

2.4.4 Birds

Birds make use of both aquatic and terrestrial habitat in and potentially affected by the Madison County Site within SEMOLMD. These areas generally fall within the Ozark Upland physiographic area, in which over 100 bird species breed (Fitzgerald et al. 2000). Special-status avian species occurring in the MCM Site include the state ranked “vulnerable” bald eagle (*Haliaeetus leucocephalus*) and the greater roadrunner (*Geococcyx californianus*). In recent years, populations of special-status species such as the northern harrier (Circus cyaneus), Swainson's hawk (*Buteo swainsoni*), and cerulean warbler (*Dendroica cerulean*) have declined. (Fitzgerald et al. 2000). Bachman’s sparrow (*Peucaea aestivalis*), a state endangered migratory bird is also at risk at the MCM Site. Other ground-feeding song birds likely to occur in Madison County that are birds of conservation concern include field sparrows, worm-eating warbler, hooded warbler, Kentucky warbler, Louisiana water thrush, northern bobwhite quail, and wood thrush. Ground-feeding birds are of particular concern due to their propensity to incidentally ingest contaminated soil, as indicated below (See Appendix B).

Beyer et al (2004) and Sileo et al (2003) documented exposure and toxic effects to migratory birds resulting from releases of mining-related heavy metals into sediments and terrestrial environments in the Tri-State Mining District. Concentrations of lead, zinc, and/or cadmium in MCM Site mill waste are comparable to concentrations in mill waste and sediment found to cause a toxic effect to migratory birds in the Beyer and Sileo studies.

A more recent study of lead toxicity to songbirds in the SEMOLMD by Beyer et al (2013) examined sites with soil lead concentrations between 1,000-3,200 mg/kg. Songbirds collected included American robins (*Turdus migratorius*) and northern cardinals (*Cardinalis cardinalis*). Earthworms were analyzed to find levels of 33–4,600 mg Pb/kg dry weight (dw) in tissues. Vermivore songbirds were likely to have toxic effects from the ingestion of these earthworms. Tissue analysis of the songbirds in the study showed mean tissue Pb concentrations in songbirds collected from the contaminated sites were greater ($p>0.05$) than those in songbirds from reference sites by factors of 8 in blood, 13 in liver, and 23 in kidney. The authors concluded that soils in the SEMOLMD contaminated by mining and smelting (with lead levels >1,000 mg/kg) are poisoning ground feeding birds.

Stratus Consulting (2014) evaluated Beyer's songbird data compared to other well documented studies on birds exposed to lead in an effort to quantify injury. Stratus developed injury 'bins' that contained ranges of soil contamination that correspond to levels of habitat injury as measured by lead in songbird tissues. The bins designated increasingly severe levels of injury to birds corresponding to increasing levels of soil lead contamination Stratus also identified a soil concentration of 345 ppm lead as a point at which injury to birds begins as indicated by delta-aminolevulinic acid dehydratase (ALAD) inhibition in SEMOLMD cardinals. An ALAD inhibition of greater than 50% is considered injury by the CERCLA NRDAR regulations (43 C.F.R. §11.62(f)(4)).

The MCM Site ERA concluded there was unacceptable risk to several model organisms representing a variety of ecological niches or feeding guilds (EPA 2006). The screening level risk assessment concluded that there was unacceptable risk from exposure to lead, zinc, and/or cadmium to vermivores and piscivores.

Birds exposed to metals at the MCM Site may also have been impacted by mining-related habitat losses (see "Vegetation" section above). Less vegetative cover and lower quality vegetative habitat mean fewer insects, fruits, and seeds for smaller birds to consume. Plants also provide food for small mammals, which in turn are the prey of larger birds. Mining-related impacts to plants therefore represent a loss of habitat that can reverberate through the food web to the highest-level predators.

2.4.5 Mammals

The Madison County Site mammals rely on both aquatic and terrestrial habitats. Mammals within the MCM Site include raccoon (*Procyon lotor*), coyote (*Canis latrans*), striped skunk (*Mephitis mephitis*), bobcat (*Lynx rufus*), black bear (*Ursus americanus*) whitetail deer (*Odocoileus virginianus*), red fox (*Vulpes vulpes*), mice, shrews, voles and various other small rodents. Special-status mammals include the Missouri-endangered plains spotted skunk (*Spilogale putorius interrupta*), and the federally-endangered gray bat (*Myotis grisescens*),

Indiana bat (*Myotis sodalis*), and the proposed-endangered northern long-eared bat (*Myotis septentrionalis*). Lack of vegetation in barren chat mining areas limits habitat and food resources for mammals. Small mammals may also be experiencing direct toxic effects from exposure to metals from mining wastes. Particularly at risk are terrestrial vermivores which ingest earthworms with gut soils as well as ingesting incidental surface soils. Region 7 (EPA 2006) took earthworm samples at two contaminated locations in MCM Site and one background sample. The results showed that the earthworms found at the contaminated sites contained between (approximately) 19-43% higher lead concentrations than the earthworms found at the background location.

2.5 CONTAMINANTS OF CONCERN

For assessment planning purposes, the Trustees will focus on cadmium (Cd), lead (Pb), zinc (Zn), copper (Cu), and nickel (Ni); these hazardous substances have significant potential for toxicity to many different natural resources. Based on existing relevant data, the Trustees know that these metals are commonly found at elevated levels in soils, sediments, and/or surface waters at the MCM Site. The Trustees recognize that other contaminants and conditions may adversely affect natural resources at the MCM Site. After reviewing results from studies conducted under the DAP, the Trustees will consider additional hazardous substances, including but not limited to, cobalt (Co), and manganese (Mn), if warranted. The following paragraphs, however, focus on the primary hazardous substances, their toxicology, and associated environmental hazards.

2.5.1 Cadmium

Cadmium (Cd) is not biologically essential or beneficial to any known living organism and is toxic to all known forms of life (Eisler 2000). Freshwater[4] animals tend to be most heavily impacted by cadmium contamination. Impacts to freshwater animals include death, reduced growth, and inhibited reproduction (Eisler 2000). In freshwater systems, the lethal effects of cadmium can be reduced by limiting exposure time and increasing water hardness[5] (Eisler 2000). Sublethal effects of cadmium in freshwater organisms include decreases in plant standing crop, decreases in growth, inhibition of reproduction, immobilization, and population alterations (Eisler 2000). Mammals and birds are comparatively resistant to the toxic[6] effects of cadmium, though exposure to high levels can be fatal (Eisler 2000).

Animals can be exposed to environmental cadmium through inhalation or ingestion. Cadmium is a known carcinogen, a known teratogen, and a probable mutagen (Eisler 2000; ATSDR 2012). Studies investigating carcinogenicity have focused on mammals. Cadmium has been shown to cause tumors in the prostate, testes, and hematopoietic (blood-related) systems in rats (ATSDR

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[4] Freshwater refers to waters that are not saline (salty).

[5] Water hardness is a measure of the content of certain naturally-occurring elements in water, especially calcium and magnesium.

[6] Toxins cause direct injury to an organism as a result of physiochemical interaction. Carcinogens cause cancer (for example, tumors, sarcomas, leukemia). Mutagens cause permanent genetic change. Teratogens cause abnormalities during embryonic growth and development.

2012). Based on studies in mice and bacteria, cadmium may be mutagenic (Ferm and Layton 1981, as cited in Eisler 2000). When present, cadmium is detected in particularly high concentrations in the leaves of plants and the livers and kidneys of vertebrates (ATSDR 2012; Scheuhammer 1987, as cited in Eisler 2000).

2.5.2 Lead

Lead is not biologically essential or beneficial to any known living organism (Eisler 2000). It can be incorporated into the bodies of individual organisms by inhalation, ingestion, absorption through the skin, and (in mammals) placental transfer from the mother to the fetus (Eisler 2000). Toxic in most chemical forms, lead negatively affects survival, growth, reproduction, development, and metabolism of most animals under controlled conditions, but its effects are substantially modified by numerous physical, chemical, and biological variables. Younger, immature organisms tend to be more susceptible to lead toxicity (Eisler 2000). When absorbed in excessive amounts, lead has carcinogenic or co-carcinogenic properties (Eisler 2000). In large amounts, it is also a mutagen and a teratogen (Eisler 2000).

It has been demonstrated that aquatic animals experience adverse effects such as reduced survival, impaired reproduction, and reduced growth (Eisler 2000). As with cadmium, increased water hardness decreases lead bioavailability to aquatic animals (Wong et al. 1978 and NRCC 1973, both as cited in Eisler 2000). Early research suggested that birds are unlikely to show adverse effects from environmental lead (except when lead objects such as shot are directly ingested); however, there is now a body of evidence linking waterfowl poisoning with ingestion of lead-contaminated sediments, especially in the Coeur d'Alene area of Idaho (Chupp and Dalke 1964, Blus et al. 1991, Beyer et al. 1998, Heinz et al. 1999, all as cited in Eisler 2000). There are few data regarding the effect of environmental lead on mammalian wildlife (Eisler 2000).

Lead also can harm plant species. Generally, large amounts must be present in soils before terrestrial plants are affected, although sensitivity varies widely among species (Demayo et al. 1982). Effects of lead toxicity in plants include reduced plant growth, photosynthesis, mitosis, and water absorption (Demayo et al. 1982).

2.5.3 Zinc

Zinc (Zn) is an essential trace element for all living organisms, and zinc deficiency in animals can cause a variety of adverse effects (Eisler 2000; ATSDR 2005). Zinc is also toxic at high concentrations, although its toxicity depends on its chemical form and other environmental parameters (Eisler 2000). Zinc is not carcinogenic, although in certain chemical forms, zinc can be mutagenic (Thompson et al. 1989, as cited in Eisler 2000). Zinc is teratogenic to frog and fish embryos, but there is no conclusive evidence of teratogenicity in mammals (Dawson et al. 1988 and Fort et al. 1989, both as cited in Eisler 2000).

Environmental effects of excess zinc can be significant at relatively low concentrations (Eisler 2000). Terrestrial plants can die from excess zinc in the soil (Eisler 2000). Freshwater animals can also experience adverse effects, including reduced growth, reproduction, and survival (Eisler 2000). Ducks experience pancreatic degeneration and death when fed diets containing high

concentrations of zinc (Eisler 2000). Mammals can generally tolerate greater than 100 times their minimum daily zinc requirement (NAS 1979, Wentink et al. 1985, Goyer 1986, Leonard and Gerber 1989, all as cited in Eisler 2000), but levels that are too high affect their survival, metabolism, and well-being (Eisler 2000).

2.5.4 Copper

Copper is an essential nutrient for most organisms. However, contamination above various concentration and bioavailability thresholds are toxic (Rainbow and Luoma, 2011). Copper is among the most toxic of the heavy metals to aquatic biota, but birds and mammals are relatively resistant to copper toxicity. Phytotoxicity caused by copper is rare in higher plants, but has been documented on mine spoils (Eisler, 1988a).

Excess copper causes altered permeability of cellular membranes and causes a reaction that creates free radicals that oxidize lipids (Aeseth and Norseth, 1986). In aquatic invertebrates copper causes gill damage and in fishes it interferes with osmoregulation (Hodson et al. 1979). Copper also interferes with energy metabolism and can reduce the activities of enzymes that regulate ATP syntheses (Hansen et al. 1992). Copper is toxic to algae and is often used in ponds and other aquatic applications as an algaecide.

Copper is toxic to mammals only at high concentrations due to their ability to excrete excess copper. However, diets high in copper fed to domestic sheep have been found to cause liver damage, impaired reproduction, and death (Eisler, 1998). Similarly, high copper diets fed to ducklings have been demonstrated to be fatal (Wood and Worden, 1973).

There is not definitive evidence that copper is carcinogenic, mutagenic, or teratogenic except under grossly elevated exposure (Eisler, 1998a).

2.5.5 Nickel

Nickel (Ni) is uniformly present in the biosphere. Nickel in the environment from natural or anthropogenic sources is transported through the system by chemical and physical processes and through biological transport mechanisms (National Academy of Sciences [NAS] 1975; World Health Organization (WHO) 1991). Nickel is an essential micronutrient for many species of microorganisms and plants and several species of vertebrates, including chickens, cows, goats, pigs, rats, and sheep (WHO 1991). The WHO classifies nickel compounds in Group 1 (human carcinogens) and metallic nickel in group2B (possible human carcinogen; WHO 1991).

Adverse effects of excess nickel are documented for bacteria, algae, yeasts, plants, protozoans, mollusks, crustaceans, insects, annelids, echinoderms, fishes, amphibians, birds, and mammals (Eisler 1998b). The majority of terrestrial plants are nickel-intolerant species and are restricted to soils of relatively low nickel content; some plants without specific nickel tolerance can accumulate nickel, but at a cost of reduced growth (Rencz and Shilts 1980). Birds, especially waterfowl feeding in nickel polluted areas, are at risk due to the high accumulation of nickel in aquatic food plants (Eastin and O'Shea 1981). In mammals, the toxicity of nickel is a function

of the chemical species of nickel, dose, and route of exposure. Toxic effects of nickel to mammals are documented for respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, dermal, ocular, immunological, developmental, neurological, and reproductive systems (NAS 1975; WHO 1991).

2.6. CONFIRMATION OF EXPOSURE[7]

The result of mining, milling and smelting activities is past and ongoing exposure of natural resources–land, water, plants and animals–to metals, potentially causing injuries to these resources and the services they provide to humans and the environment. The Trustees intend to investigate and document these losses through the studies set forth in this Assessment Plan.

A substantial body of information is already available demonstrating past and ongoing exposure of the MCM Site natural resources to hazardous substances as evidenced below.

2.6.1 Surface Water

Metal concentrations, particularly those of zinc and lead, have exceeded the ambient water quality criteria (AWQC) in samples collected by USEPA in 2006 at Sweetwater Branch, Harmony Lake tailings, Spiva Branch, and an unnamed creek on the north end of the Anschutz Property. Sediment concentrations of metals in waterways of the MCM Site exceed published toxicity benchmarks for the protection of aquatic life (MacDonald et al. 2000). The highest concentration of Pb in sediments was found in the Little St. Francis River near the Little St. Francis River Chat pile (EPA 2011a).

2.6.2 Geologic Resources

EPA's work on the MCM Superfund Site has documented high concentrations of contaminants in mine wastes and nearby soils at levels that exceed both national average soil concentrations and concentrations toxic to vegetation (EPA 2011a).

2.6.3 Groundwater

Studies have found concentrations of metals in the shallow aquifer that are higher than background concentrations by up to an order of magnitude and that exceed ground water criteria (Black & Veatch 2008). These wells have shown an unacceptable risk if used as a drinking water source and may be a risk to non-human receptors as well. EPA is planning further studies of the groundwater surrounding the chat/tailings piles at the MCM Site.

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[7] The CERCLA NRDAR regulations require that exposure of at least one of the natural resources identified as potentially injured "has in fact been exposed to the released substances" (43 CFR §11.37(a)). This Plan confirms that a variety of potentially-injured resources have been exposed to hazardous substances, including cadmium, lead, and zinc.

2.6.4 Biotic Resources

The 2006 ERA for MCM Site showed elevated levels of lead in the tissues of fish collected in Mill Creek and Little St. Francis River along with a decrease in the abundance of some sensitive species of benthic macroinvertebrates. Toxicity tests indicate that sediments from the Little St. Francis River and its tributaries pose an acute and chronic toxicity risk to benthos. There is evidence supporting of phytoxicity to terrestrial plants in mine waste areas. Also, according to the 2006 ERA, there is a potential hazard to woodcocks, shrews, earthworms, blue herons, and vermivores.

Altogether, these data confirm that natural resources in the MCM Site have been, and continue to be, exposed to elevated levels of metals resulting in injuries to natural resources.

2.7 PRELIMINARY DETERMINATION OF RECOVERY PERIOD[8]

Recovery period is defined under 43 C.F.R. §11.14(gg) as "either the longest length of time required to return the services of the injured resource to their baseline condition, or a lesser period of time selected by the authorized official and documented in the Assessment Plan." Several factors can influence estimates of recovery time, including ecological succession patterns, growth or reproductive patterns, life cycles, ecological requirements of plants and animals (including their reaction or tolerance to the hazardous substances involved), biological recruitment potential, the bioaccumulation and extent of hazardous substances in the food web and the chemical, physical and biological removal rates of the hazardous substances.

As noted in previous sections of this Addendum, substantial mining activities in the MCM Site were undertaken for more than a century, and measurements of metals in the environment demonstrate that these contaminants have been present at levels associated with adverse impacts to natural resources for decades.

Data from similar sites in other locations, and research presented in the technical literature, suggest a recovery period on the order of at least decades in the absence of active remediation or restoration efforts beyond those already implemented or planned. Metals are elements and may change their chemical form or become dispersed in the environment, but they do not break down or degrade. Elevated levels of metals have been and continue to be present in a wide variety of natural resources within the MCM Site. Available information suggests that natural processes will take a very long time to remove the contamination or render it biologically unavailable, given the amounts present and the environmental processes involved.

The Trustees recognize that implemented or planned actions through Superfund or other programs may hasten the recovery of some resources at the MCM Site. However, information currently available to the Trustees indicates that planned or implemented actions are not

[8] The CERCLA NRDAR regulations require than an assessment plan include a preliminary estimate of the time needed for injured resources to recover (43 CFR §11.31(a)(2)).

sufficient in scope or design to change the preliminary finding that adverse mining-related impacts to natural resources in the MCM Site are likely to persist for decades or longer.

2.8 QUALITY ASSURANCE MANAGEMENT

The CERCLA NRDAR regulations require that the Trustees develop a Quality Assurance Plan (QAP) that "satisfies the requirements listed in the NCP and applicable EPA guidance for quality control and quality assurance plans." 43 CFR §11.31(c)(2). A QAP is needed to ensure the validity of data collected as part of the NRDAR and to provide a solid foundation for the Trustees' subsequent decisions. Also relevant to this effort are the FWS guidelines developed under the Information Quality Act of 2001. All information developed in this NRDAR will be in compliance with these guidelines. Please refer to Final Phase I Damage Assessment Plan for Southeast Missouri Lead Mining District: Big River Mine Tailings Superfund Site, St. Francois County and Viburnum Trend Sites, Reynolds, Crawford, Washington, and Iron Counties (Mosby et al. 2009) for more detailed information regarding quality assurance practices.

CHAPTER 3 OVERVIEW OF CURRENTLY PROPOSED AND/OR CONTEMPLATED STUDIES.

At this time, one assessment study is planned for the MCM Site, with another study in the development stage. The crayfish population and in situ toxicity study, a draft of which is included as Appendix B for public review and comment, is planned for spring and summer 2015. The transition zone study plan is currently in development. Once a draft has been completed, it will be made available to the public for review and comment. The transition zone study has not been scheduled, but it is anticipated that field work will begin in the fall of 2015 and be completed by the end of 2015.

The crayfish study will evaluate crayfish density and other population metrics in riffle habitat at various streams impacted by OU-2. A second phase of the study will place caged crayfish in a subset of study streams to serve as an in-situ toxicity evaluation. These results will be compared to reference streams and stream reaches that drain other upstream mining areas that could be used to establish baseline conditions. A draft study plan is contained in Appendix B.

The transition zone soil study will be designed to characterize the scope of soil contamination surrounding mine and mill dumps in OU-2. This study will be conducted using XRF meters to record metals concentrations in transects leading away from mine or mill waste. A subset of the samples will be submitted for confirmatory laboratory analyses.

Other potential studies could include migratory bird exposure study, small mammal exposure, food web analyses, and groundwater injury quantification.

Injury data collected through studies discussed above will be used to establish pathway determinations, which describes fate and transport of contaminants and food-web uptake. The data collected through these studies will also support injury quantification—the extent and severity of injury. This injury quantification information will then be used to determine the

amount of compensation owed to the public for the loss of, destruction of, and injury to natural resources and their associated services in the subsequent phase of the NRDAR process.

REFERENCES

Aaseth, J. and Norseth, T.. 1986. Copper. Pg 233-254 in Handbook on the Toxicology of Metals, 2nd ed. Volume II: specific metals. Friberg, L. Nordberg, G.F. and Vouk, V.B. editors, Elsevier, New York.

ATSDR (Agency for Toxic Substances & Disease Registry). 2012. Toxicological Profile for Cadmium.

ATSDR (Agency for Toxic Substances & Disease Registry). 2005. Toxicological Profile for Zinc.

Beyer, W.N., J. Dalgarn, S. Dudding, J.B. French, R. Mateo, J. Miesner, L. Sileo, and J. Spann. 2004. Zinc and lead poisoning in wild birds in the Tri-State Mining District (Oklahoma, Kansas, and Missouri). Archives of Environmental Contamination and Toxicology: 48(1)108-117.

Beyer, W. N., J. C. Franson, J. B. French, T. May, B. A. Rattner, V. I. Shearn-Bochsler, S. E. Warner, J. Weber, and D. Mosby. 2013. Toxic exposure of songbirds to lead in the southeast Missouri lead mining district. Archives of Environmental Contamination and Toxicology 65(3):598-610..

Black & Veatch. 2005a. Draft site description report: Big River Mine Tailings Site, St. Francois Count Missouri. Work Assignment No. 093-RICO-07CR. Black & Veatch Project No. 046139.01.01 April 2005 Revision 1. Prepared for the U.S. EPA, Region VII.

Black & Veatch. 2005b. Draft Site Inspection Report. Big River Mine Tailings Site. Prepared for USEPA, Region VII.

Black & Veatch. 2008. Final Madison County Mines Site Remedial Investigation Report, Madison County, Missouri. Prepared for USEPA, Region VII.

CH2M Hill, Black & Veatch, ICF, PRC, and Ecology and Environment. 1987. Assessment of the toxicity of arsenic, cadmium, lead and zinc in soil, plants, and livestock in the Helena Valley of Montana for East Helena Site (Asarco), East Helena, Montana. Reclamation Research Unit, Montana State University, Bozeman, MT. Prepared for U.S. EPA Hazardous Site Control Division Contract No. 68-01-7251, 206 p.

Dames & Moore. 1990. Madison Mine Preliminary Site Characterization Report. April 3.

Dames & Moore. 1993a. Final Ecological Risk Assessment for Cherokee County, Kansas, CERCLA Site: Baxter Springs/Treece Subsites. March 24.

Demayo, A., M.C. Taylor, K.W. Taylor, and P.V. Hodson. 1982. Toxic effects of lead and lead compounds on human health, aquatic life, wildlife plants, and livestock. CRC Crit. Rev. Environ. Control 12:257-305.

Eastin, W. C., Jr., and T. J. O'Shea. 1981. Effects of dietary nickel on mallards. Journal of Toxicology and Environmental Health 7:883-892.

Eisler, R. 1998(a). Copper hazards to fish, wildlife, and invertebrates: a synoptic review. Biological Science Report USGS/BRD/BSR-1997-0002. Contaminant Hazard Reviews. Report No. 33

Eisler, R. 1998(b). Nickel hazards to fish, wildlife, and invertebrates: a synoptic review. Biological Science Report USGS/BRD/BSR-1998-0001. Contaminant Hazard Reviews. Report No. 34

Eisler, R. 2000. Handbook of Chemical Risk Assessment: Health Hazards to Humans, Plants, and Animals. Volume1: Metals. Lewis Publishers, Boca Raton, FL.

EPA. 2006. Madison County Mine Site Ecological Risk Assessment Final Report, May 2006.

EPA. 2011a. Supplemental Remedial Investigation Report for Madison County Mines Superfund Site, Madison County, Missouri.

EPA. 2011b. Proposed Plan Conrad Tailings Operable Unit 4, Madison County Mines Superfund Site, Madison County, Missouri.

EPA. 2014. Record of Decision Madison-Wide Residential Operable Unit 03, Madison County Mines Superfund Site, Madison County, Missouri.

Fargasova, A. 2001. Phytotoxic effects of Cd, Zn, Pb, Cu, and Fe on *Sinapis alba* L. seedlings and their accumulation in roots and shoots. Biol. Plant 44 (3):471-473.

Ferm, V. H., and W. M. Layton, Jr. 1981. Teratogenic and mutagenic effects of cadmium. Pages 743-756 *in* J. O. Nriagu (ed.). Cadmium in the environment. Part 2, Health effects. John Wiley, New York.

Fitzgerald, J., and D.N. Pashley. 2000. Partners in Flight Bird Conservation Plan for The Ozark/Ouachitas (Physiographic Area 19). Version 1.0. Viewed 4-22-08 at http://www.partnersinflight.org/bcps/plan/pl_19_10.pdf

Hansen, J.I. T. Mustafa, and M. Depledge. 1992. Mechanisms of copper toxicity in the shore crab, *Carcinus maenas*. II. Effects on key metabolic enszymes, metabolites and energy charge potential. Marine Biology 114:259-264..

Hodson, P.V. U. Borgmann, and H. Shear. 1979. Toxcicity of copper to aquatic biota. Pg. 307-372 in Copper in the Environment. Part 2:health effects. J.O. Nriagu editor. John Wiley, New York.
.

Horner Shifrin. 2014. City of Fredericktown Drinking Water Reservoir Engineering Report, Fredericktown, Missouri.

Kapustka, L. A. Technical Memorandum - Expert Opinion Regarding Phytotoxicity Due to metals in the soils of the Tri-State and Eastern Missouri Lands Owned or Operated by Asarco, Inc. and the subject of US Department of Justice Case 05-21207. July 2007.

Kloke, A., D.R. Sauerbeck, and H. Vetter. 1984. The contamination of plants and soils with heavy metals and the transport of metals in terrestrial food chains. In: Changing Metal Cycles and Human Health. J.L. Nrigau (ed.). Dahlem Konferenzen, Berling, Heidelberg, New York, Tokyo: Springer-Verlag.

MacDonald, D.D., C.G. Ingersoll, and T.A. Berger. 2000. Development and Evaluation of Consensus-Based Sediment Quality guidelines for Freshwater Ecosystems. Archives of Environmental Contamination and Toxicology 39(1):20-31.

Missouri Department of Conservation. 2014. Natural Heritage Database. http://mdc.mo.gov/your-property/greener-communities/heritage-program.
Mosby, D. Personal Communication. June 27, 2007. U.S. Fish and Wildlife Service.

Mosby, D. E., J.S. Weber, and F. Klahr. 2009. Final Phase I Damage Assessment Plan for Southeast Missouri Lead Mining District: Big River Mine Tailings Superfund Site, St. Francois County and Viburnum Trend Sites, Reynolds, Crawford, Washington, and Iron Counties.

National Academy of Sciences (NAS). 1975. Medical and biological effects of environmental pollutants. Nickel. National Research Council, National Academy of Sciences, Washington, D.C. 277 pp.

Nigh, T.A. and W.A. Shroeder. Atlas of Missouri Ecoregions. Missouri Department of Conservation.

Pahlsson, A.M. 1989. Toxicity of heavy metals (An, Cu, Cd, Pb) to vascular plants: a literature review. Water Air Soil Pollut. 47:287-319.

Reed, A.W., G.A. Kaufman, and D.W. Kaufman. 2004. Influence of fire, topography, and consumer abundance on seed predation in tallgrass prairie. Canadian Journal of Zoology 82:1459-1467.

Rainbow, P.S., Luoma, S.N. 2011. Trace metals in aquatic invertebrates, in Environmental Contaminants in Biota, Interpreting Tissue Concentrations, 2nd ed. Beyer, W.N., Meador, J.P. editors, CRC Press, Boca Raton, FL

Rencz, A. N., and W. W. Shilts. 1980. Nickel in soils and vegetation of glaciated terrains. Pages 151-188 in J. O. Nriagu, editor. Nickel in the environment. John Wiley & Sons, New York.

Sileo L, Beyer WN, and Mateo R. 2003. Pancreatitis in wild Zn-poisoned waterfowl. Avian Dis 32:655–660.

Stratus Consulting. 2014. Associating soil lead with adverse effects on songbirds in the Southeast Missouri Mining District. July 28, 2014.

Struckhoff, M.A., E.D. Stroh, and K.W. Grabner. 2013. Effects of mining-associated lead and zinc contamination on native floristic quality. Journal of Environmental Management 119:20-28.
United States Fish and Wildlife Service and Missouri Department of Natural Resources.2014. Preassessment Screen and Determination Madison County Mines Site, Madison County, Missouri.

United States Geological Survey (USGS). 2006-2007. USGS Water-Quality Data for the Nation. Online. Accessed June 2008. http://waterdata.usgs.gov/nwis

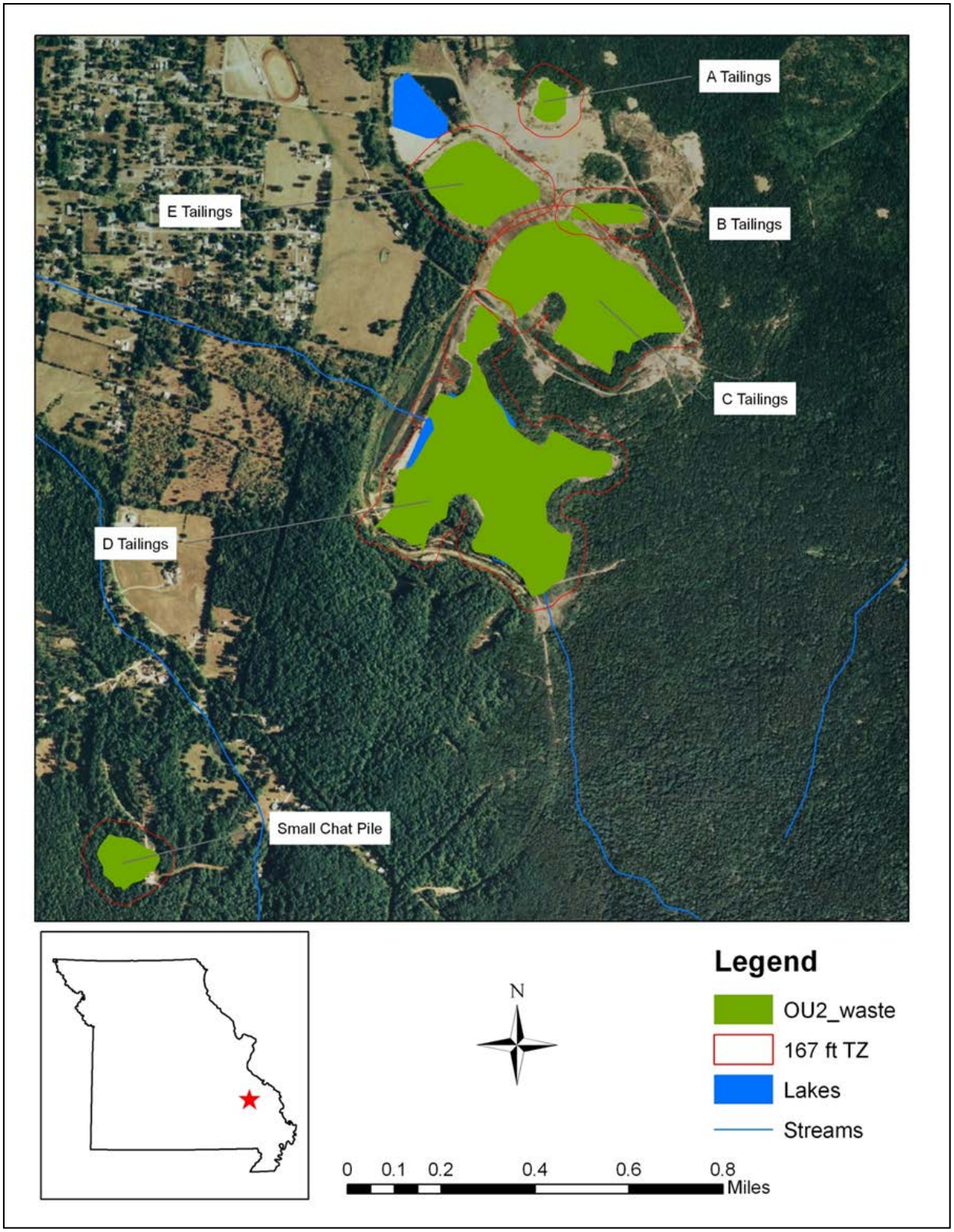
Wood, E.C. and A.N. Worden. 1973. The influence of dietary copper concentration on hepatic copper in the duckling and the chick. Journal of the Science of Food and Agriculture 24:167-174.

World Health Organization (WHO). 1991. Nickel. Environmental Health Criteria 108. 383 pp.

APPENDIX A

Map of Madison County Mines Site OU2

Figure 1. Map of Madison County Mines Site showing mine waste of OU2 with transition zone buffer of 167 feet.



APPENDIX B

DRAFT Crayfish Population and In Situ-Toxicity Study Plan

CERC Research Study Plan Title: Madison County Mines Site Resource Damage Assessment: Crayfish populations and in-situ toxicity testing of crayfish in the Little St. Francis River drainage

CERC Tracking # (provided when proposal submitted):

BASIS+ Project/Task/Subtask Number: SB00C2G Task 2

Date Research Study Plan Prepared: April 15, 2015

Date Research Study Plan Revised:

USGS/BRD Center: Columbia Environmental Research Center

Facility Contact: Jo Ellen Hinck

Project Contact: Ann L. Allert, William G. Brumbaugh, Danielle Cleveland, Robert J. DiStefano (Missouri Department of Conservation)

Date Initiated: March 1, 2015

I. **Rational and Justification**:

Southeast Missouri Lead Mining District (SEMOLMD) has been mined for lead-zinc ore for about 300 years. Metals, including lead, zinc, copper, cadmium, cobalt, and nickel were exploited to various degrees. Extraction and processing ore left a legacy of elevated concentrations of metals in stream sediment, water, and biota. The Madison County Mines (MCM) Superfund Site, located near Fredericktown, Missouri, is the southeastern end of the SEMOLMD. The principle drainage system for the MCM site is the Little St. Francis River and its tributaries. Contaminated areas in the MCM (e.g., chat, tailings, and contaminated soils) continue to be potentially primary sources of metals to the aquatic biota in the Little St. Francis River (USFWS 2015). Contaminated sediment provides exposure through incidental ingestion and by increasing metals concentrations in pore water of the sediment. Groundwater and surface water are also exposure paths for aquatic biota. Sediment metals concentrations in the Little St. Francis River have been found to be high enough to cause adverse effects to aquatic communities (Wooster-Brown 2006).

Crayfish are important to the ecology and economies of the Ozarks (DiStefano 2005). They eat/process live, dead and decaying plants as well as animal matter, and dominate invertebrate biomass in Ozark streams. They are prey for more than 200 animals, and are the primary food of popular sport fishes (e.g., smallmouth bass, rock bass, shadow bass; DiStefano 2005; Hobbs 1993). Crayfish have been identified as a critical ecosystem component of intermediate trophic position that may provide critical roles in facilitating both upward and downward flow of nutrients and energy (Momot 1995; Rabeni et al. 1995). It is likely that loss of crayfish populations in some mining-impacted areas leads to decreased rates of detrital decomposition thereby affecting functional processes in the

Ozark Plateau (Allert, unpublished data). Leachates from tailings piles and soils enter groundwater supplies for Ozark streams and have led to contaminated aquatic food chains and loss of biota including crayfish and sculpin (Allert et al. 2008, 2009a, 2009b, 2013, 2013; Besser et al. 2007). In-stream studies have revealed that crayfish are absent from locations immediately adjacent to lead mining operations and caged studies of crayfish have demonstrated a significant inverse relationship between survival of juvenile crayfish and dissolved trace metals in pore-water in the SEMOLMD and other mining districts in Missouri (Allert et al. 2008, 2009a, 2012, 2013).

II. **Objectives** (What):

We propose to conduct a two-tier investigation that includes a survey of crayfish populations and in-situ toxicity tests using endemic crayfish in the Little St. Francis River watershed in Madison County. Two candidate crayfish species for federal listing, *Orconectes quadruncus* and *Orconectes peruncus*, are found in the watershed and may be potentially impacted by metals contamination. Recent studies have demonstrated that the loss of endemic crayfish species alters macroinvertebrate structural and functional communities (Charlebois and Lamberti 1996; Freeland-Riggert 2014; Hanson et al. 1990). Data provided in these studies can be used to evaluate whether metals contamination in the Little St. Francis River cause injury to aquatic organisms and to wildlife, including migratory birds, which depend on those aquatic organisms. Analytical samples will be taken in support of biological studies. Trace metals concentrations in surface water, pore water, sediment, and biota (crayfish, fish, and other macroinvertebrates) will provide information to assess exposure of metals through food-web pathways.

III. **Listing of Studies:**

A. **Study 1**

1. **Principal Investigator(s):** Ann L. Allert, William G. Brumbaugh, Danielle Cleveland, Robert J. DiStefano (Missouri Department of Conservation)

2. **Specific Objectives:** a) Determine crayfish riffle/run densities and crayfish species composition in the Little St. Francis River drainage of southeast Missouri; b) measure selected metals (Pb, Zn, Cd, Co, Ni, Cu) concentrations in surface water, sediment, and crayfish as measures of metals exposure; c) characterize physical habitat in riffles/runs used by crayfish and water quality conditions of the Little St. Francis River drainage; and d) evaluate relationships among crayfish riffle/run densities, concentrations of mining-derived metals in water, sediment, and crayfish, and other water and physical habitat characteristics.

3. **Experimental Design or Methodological Approach:** Sites to be sampled will be selected based on data collected in previous studies that characterized metals concentrations in water, sediment, and animal tissue (MDNR 1986, 2004, 2005, 2010; USEPA 2006) and from pre-assessment X-ray fluorescence (XRF) meter data

collected for metals concentrations in sediment. We propose that eight sites be distributed within the Little St. Francis River drainage that are upstream, directly downstream, and remotely downstream of Operable Unit 2 (OU2; i.e., tailings or chat piles, mines).

Crayfish density: A maximum of eight sites will be sampled. If possible, two sites will be located upstream of the mining impact area and/or in a tributary(ies) of the Little St. Francis River without mining-sourced material. If possible, sites will be selected which have 1–4 riffle/run complexes. At each site, quantitative crayfish samples will be collected within 1–4 riffle/run complexes.

Crayfish will be sampled in riffle/runs by disturbing the substrate inside a 1-m^2 weighted polyvinyl chloride (PVC) quadrat frame placed on the stream bottom directly upstream of a kick seine (1.5 m length by 1.5 m height) with a 3-mm diameter delta mesh (Allert et al. 2012; DiStefano et al. 1993, 2003, 2009a; Williams et al. 2014). Sampling will begin at downstream ends of riffles and proceed upstream. At each site, a total of 21 kick samples will be obtained by distributing 21 samples between 1–4 riffle/runs at that site. Crayfish collected will be identified to species (Pfleiger 1996), examined to determine sex, measured for carapace length (from the tip of rostrum to the posterior edge of the cephalothorax to nearest 0.1 mm), and released. Voucher specimens and unidentifiable crayfish will be placed on ice, returned to Columbia Environmental Research Center (CERC) for identification and archived in the walk-in freezer.

Crayfish trace metals:, At each site, we propose to take specimens of *O. quadruncus* collected during the population study at random for metals analysis. Three replicate composites of 3–5 crayfish will be taken at each site; one from each of the riffle/runs sampled. ***Individuals taken for metals analyses should be the same species for all riffles and sites and will be identified on datasheet (Appendix 1). In addition, crayfish should be similar in size across all riffles and sites.*** If more than one species is required per riffle or site for the metals samples because of the low number of individuals of the target species, only one species should be placed in each sampling jar. All samples for metals analyses will be placed in pre-cleaned jars, stored on ice until they are returned to CERC, where they will be frozen until preparation for analyses. Whole crayfish will be freeze-dried (SOP P.259) and cryogenically pulverized to a powder-like consistency (SOP P.213). Sub-samples (0.25 g dry) will be digested using a mixture of concentrated nitric and hydrochloric acids and with heating in a laboratory microwave oven (SOP P.636). The digestates will be analyzed using inductively-coupled plasma mass spectrometry (ICP-MS) for Pb, Zn, Cd, Ni, Co, and Cu (SOP P.241; Allert et al. 2008; Appendix 2).

Habitat measurements: Sites will be identified using a Thales® Mobile Mapper global positioning system (GPS) receiver. Current velocity and depth will be measured at each crayfish sampling location (i.e., kick seine quadrat), and along transects set across each riffle using Marsh McBirney® flow meter or a Hach® FH950.0 flow meter and depth rod. Substrate composition will also be assessed using

visual methods at each crayfish seining location (Appendices 3 and 4). Stream discharge will be calculated at each site. When possible, we will determine or calculate selected landscape variables such as watershed area, stream order, land use area, and area of mining-related materials using geographic information system (GIS) or other mapping tools.

Surface water: general water quality: Surface water quality analyses (i.e., temperature, pH, conductivity, dissolved oxygen, turbidity) will be measured in situ three times (in each riffle, if present) within a site with a multi-parameter water quality instrument (i.e., Hydrolab® Quanta). A sub-surface water grab sample three times (in each riffle, if present) within a site will also be collected for additional water quality (i.e., alkalinity, hardness, ammonia, total nitrogen, total phosphorus, particulate organic carbon [POC], total suspended solids [TSS] APHA 2005), major cations (Na, K, Mg, Ca, Fe, Mn, Sr) by ICP-MS (SOP P.241; anions (F, Cl, NO_2/NO_3, Br, SO_4, PO_4, by ion chromatography; SOP P.705; USEPA Method 300); and dissolved organic carbon by combustion and infrared detection; SOP P.722; USEPA Method 415.2). See Appendices 5 and 6.

Surface water: trace metals: From each surface water sample, an aliquot will be taken for metals analyses using a polypropylene syringe and will be filtered through a 0.45-µm pore-size polyethersulfone membrane housed in a polypropylene cartridge (SOP P.566). Filtered samples will be transferred to polyethylene bottles, stored on ice, and acidified to pH <2 with Ultrex® nitric acid within 96-hr of collection (Brumbaugh et al. 2007; May et al. 1997; Appendix 5). Filtration blanks will be taken at the time of sample collection.

Sediment trace metals and carbon: At each site, a sediment sample will be taken for bulk metals analysis (Brumbaugh et al. 2007; Besser et al. 2009). Sediment samples will be sieved using a 2-mm sieve bucket in the field and a 250-µm sieve in the laboratory. Samples of both fractions from each site will be analyzed using ICP-MS for Pb, Zn, Cd, Ni, Co, and Cu following a total recoverable digestion method (SOP P.636; Brumbaugh et al. 2007). A subsample of both sediment fractions from each site will be analyzed for inorganic and total carbon (APHA 2005). The organic fraction will be determined by subtraction.

B. **Study 2**

1. **Principal Investigator(s):** Ann L. Allert, William G. Brumbaugh, Danielle Cleveland, Robert J. DiStefano (Missouri Department of Conservation)

2. **Specific Objectives:** Evaluate growth and survival of young crayfish using in-situ cages in relation to metals exposure.

3. **Experimental Design or Methodological Approach:**

Cage deployment: We will deploy cages at four sites to investigate associations between survival and growth of crayfish with metals exposure. Crayfish (preferably *Orconectes luteus* or *O. quadruncus*; Pflieger 1996) will preferably be collected as eggs of gravid females from uncontaminated sites in either the Little St. Francis River or the St. Francis River watershed. Sites used to collect brood stock will not be the same sites used in the population study or in-situ toxicity study. Coordinates of site locations of brood stock will be recorded. Females with egg masses will be transported to CERC for egg hatching and larval grow-out. Juvenile crayfish (approximately 10-mm carapace length) will be deployed in cages for a period of approximately 56 days. Proposed sampling dates for metals samples are days 0, 28, 56. Cages will be checked weekly for external biofouling and position in stream.

Cage design: Environmental conditions (primarily depth) will determine the type of cage used. Cages will be made of 3-mm mesh stainless steel cloth formed into a 16 x 36 x 7-cm box (Allert et al. 2008). Cages will contain 10 crayfish, leaves, rock refugia, and supplemental food (Allert et al. 2008, Whitledge and Rabeni 1997). Rocks (i.e., coarse gravel to small cobble collected from each site-specific location) and leaves will be wrapped within 6-mm mesh bags secured at the bottom of each cage with stainless steel wire. Cages will be placed in habitats with adequate depth, most likely runs or pools in close proximity to riffles sampled for crayfish density or abundance. The bottom of cages will be placed on or below the substrate surface to expose crayfish to pore water and allow access to benthic macroinvertebrates.

Leafpacks will be a source of food and habitat for caged crayfish (Momot 1995). Leaves of five tree species [black willow (*Salix nigra*), sycamore (*Platanus occidentalis*), cottonwood (*Populus fremontii*), white oak (*Quercus alba*), and shagbark hickory (*Carya ovata*)] will be pre-leached, dried at 100–105 °C and weighed (Fairchild et al. 1987). A pre-determined amount of leaves (50 g dry weight for stainless cages) will be added to each cage.

Detrital trace metals: Some leaves will be taken at day 0, 28, and 56 for metals analyses. After sampled leaves are dried and weighed, they will be ground for metals analyzes. Leaves will be digested using concentrated nitric acid and microwave heating and analyzed by ICP-MS for Pb, Zn, Cd, Ni, Co, and Cu (Allert et al. 2008; Appendix 2).

Crayfish trace metals: Three replicated samples consisting of 5–10 crayfish from the stock of cultured young-of year crayfish will be sampled prior to stocking of crayfish into cages near (<3 days) day 0 for metals concentrations. Crayfish will be measured for carapace length (from the tip of rostrum to the posterior edge of the cephalothorax to nearest 0.1 mm), and weighed prior to being placed in pre-cleaned containers. Voucher specimens will be archived in a walk-in freezer at CERC.
At days 28 and day 56, all crayfish within cages (n =3 per sampling date) will be measured for carapace length, and weighed then placed into a pre-cleaned jar for metals analyses. Composite samples will contain 1–10 crayfish per jar, depending on the survival of crayfish in each cage. The number of replicates per day per site =3. All

samples for metals analyses will be placed in pre-cleaned jars, stored on ice until they are returned to CERC, where they will be frozen until preparation for analyses. Whole crayfish will be freeze-dried (SOP P.259) and cryogenically pulverized to a powder-like consistency (SOP P.213). Sub-samples (0.25 g dry) will be digested using a mixture of concentrated nitric and hydrochloric acids and with heating in a laboratory microwave oven (SOP P.636). The digestates will be analyzed using ICP-MS for Pb, Zn, Cd, Ni, Co, and Cu (SOP P.241; Allert et al. 2008; Appendix 2).

Fish trace metals: If stainless steel cages are used, fish (i.e., largescale stonerollers, *Campostoma oligolepis*, Pfleiger 1997) will be collected from each cage site using seines, and if necessary, backpack electroshockers. Fish will be identified on site and total length (mm) of fish will be measured. Approximately 10 fish from each site will be placed in pre-cleaned plastic bags or containers, and kept on ice during transport to CERC. Fish will be kept frozen at CERC until and after processing.

Fish will be minced, homogenized and aliquots (n =3) from each site will be separated for residue analysis. Fish tissues will be digested using concentrated nitric acid and microwave heating and analyzed by ICP-MS for Pb, Zn, Cd, Ni, Co, and Cu (Allert et al. 2009a, 2012). Minced fish for crayfish food will be placed in pre-cleaned containers and kept frozen until placed into cages. Crayfish will be fed the minced fish weekly at a ration of at least 5% crayfish body weight (Appendix 2).

Voucher specimens of fish will be placed in 10% formalin and returned to CERC for identification. Fish will be transferred to 80% ETOH after two weeks.

Macroinvertebrate trace metals: Invertebrates make up a significant portion of the diet of young-of-year crayfish (Whitledge and Rabeni 1997). An invertebrate sample will be collected from leaf material remaining in each cage (n =3 per sampling date per cage per site) or in habitats with coarse substrate at each site using a kick net or seine (n =3 per sampling date per site). Targeted organisms will include a range of macroinvertebrates. Samples will be collected on days 0, 28 and 56. Samples will be kept frozen until metals analyses. Macroinvertebrates will be digested using concentrated nitric acid and microwave heating and analyzed by ICP-MS for Pb, Zn, Cd, Ni, Co, and Cu (Allert et al. 2009a, 2012; Appendix 2).

Surface-water water quality and trace metals: Water quality monitoring and samples will be taken on days 0, 28 and 56 at each cage site. Surface water quality analyses (i.e., temperature, pH, conductivity, dissolved oxygen, turbidity) will be measured in situ at each riffle within a site with a multi-parameter water quality instrument (i.e., Hydrolab® Quanta). A surface water grab sample from each riffle within a site will also be collected for additional water quality (i.e., alkalinity, hardness, ammonia, total nitrogen, total phosphorus, particulate organic carbon [POC], total suspended solids [TSS], chlorophyll *a* [chl *a*], and major cations (Na, K, Mg, Ca, Fe, Mn, Sr) by ICP-MS and anions (F, Cl, NO_2/NO_3, Br, SO_4, PO_4) by ion chromatography (APHA 2005; Appendices 4 and 5) will be collected. In-stream water quality conditions will be monitored using YSI® multi-parameter units

(temperature, pH, conductivity, dissolved oxygen) and Onset® TempPro units (temperature).

Pore-water water quality and trace metals: We propose to collect pore water using sediment "peepers" in riffle habitats near locations where the cages are deployed. Peepers will be constructed of LDPE 24-mL containers with a 0.45-µm polyethersulfone filter at the top of the container (Brumbaugh et al. 2007; Appendix 7). Peepers will be filled with deoxygenated ultrapure water and transported in 2-L polyethylene bottles filled with deoxygenated ultrapure water. Three peepers will be deployed at each site for analysis of metals and two for measurement of pore-water quality parameters. Peepers will be buried 6–10 cm in the sediment for approximately 14 days, beginning on days 1, 14, 28, and 42. Pore-water samples for metals analyses will be collected directly from the peeper. After retrieval, it will be sealed tightly in a pre-labeled zip-seal plastic bag, and transported on ice to CERC. Samples will be acidified to pH <2 with Ultrex® nitric acid within 96 hrs.

At some locations where cobble habitats might make burial deployment of peepers impractical, a push-point (also known as a drive point) probe may be used. The probe is a narrow stainless steel sealed tube having six small slits cut into the bottom over a 2-cm length. The tube is lined with FEP tubing and will have a polypropylene mesh pre-filter sleeve fitted over the bottom end before inserting into the sediment. The probe will be driven into the substrate to a depth of about 4 cm, and a 20-mL sample of pore water will be drawn by using syringe that is attached to the FEP tubing housed inside the probe tube. Once drawn, the sample will be immediately filtered through a 45-µm polyethersulfone filter.

Pore-water quality (i.e., temperature, pH, conductivity, dissolved oxygen, alkalinity, hardness, and ammonia) will be measured (APHA 2005).

4. Listing of SOP Numbers and Titles: Requirement for analyses, sample matrices, parameters, and standard operating procedures are listed in Tables 2–4, and SOP numbers and titles are listed in Appendix 8.

5. Listing of Critical Data: Collection location (including latitude and longitude determined by GPS); date; time; physical variables (i.e., current velocity, depth, substrate particle size); water quality; quantitative metals analyses of water, crayfish and detritus, and crayfish density; and caged crayfish survival and growth (Tables 5–6).

6. Statistical Treatment: Data will be analyzed using Release 9.4 of the Statistical Analysis System. Data will be analyzed for normality, and appropriate statistical transformations will be made, if needed. Summary statistics for each endpoint (Table 2) will be computed and compared using parametric and non-parametric methods. Analysis of variance, linear regression, bivariate correlation, and multivariate techniques will be conducted to ascertain the nature of relationships among variables.

7. Acceptance or Rejection Criteria for Results: Each endpoint will have its own quality assurance program that includes standards, reference materials, and blanks. Data outside the range of acceptable criteria will be clearly noted and discussed. See Tables 2–3 for additional quality assurance information.

8. Special Safety Requirements: Gloves are advised protection against infectious agents and parasites while handling crayfish and fish. A first aid kit will be present in all field vehicles. Gloves, lab coats/plastic aprons, and protective eye-ware should be worn during the processing of water, sediment, and biological samples. Department of the Interior (DOI) regulations state that all personnel should wear floatation devices when near water. Fish could potentially be collected by electrofishing; all electrofishing and watercraft safety regulations and guidelines apply. A DOI-Certified Electrofishing Team Leader must be present during all electrofishing operations. Red Cross-Certified First Aid/CPR personnel must be present during all field collections. All USGS personnel and/or contractors who potentially could drive a government vehicle must complete the National Safety Council Defensive Driver's Training Course.

9. Animal Care and Use Requirements: All personnel involved in research activities involving live organisms must adhere to the CERC Animal Welfare Plan (AWP), and implement the spirit and intent of the policies and regulations that assure humane and ethical treatment of research animals. The CERC Animal Welfare Plan outlines the Center's strategy for compliance with the AWP and associated amendments, principles and guidelines, and it is applicable to all laboratory and field research investigations using fish and other vertebrate species. We will comply with all CERC guidelines for the humane treatment of the test organisms during culture and experimentation. Animal care SOPs are listed in Table 4 and Appendix 8.

Ice is not commonly approved to euthanize fish; however, body size and species-specific thermal tolerance should be considered when assessing the effectiveness and appropriateness of ice or an ice-slurry for the humane killing of fishes (Blessing et al. 2010; Wilson et al. 2009). Despite current guidance documents, Wilson et al. (2009) showed ice had a faster time to death and fewer signs of distress in small fish. The target species (either Largescale Stoneroller, *Campstoma anomalum* or Central Stoneroller, *Campostoma oligolepis*) are considered to be warm-water fish (e.g., avoids water temperatures <9 °C; Wismer and Christie 1987) and are small-bodied fish (average size <90 mm). Given this, we feel that the use of ice will be a humane, effective, and safe method of euthanasia.

We will have a contingency plan to use tricaine methanesulfonate (MS222) as our chemical anesthetic (dose >250 mg/L) on collected fish, if ice proves to be ineffective. Concentration solutions will be packaged securely and returned to CERC for disposal.

10. Biosecurity Requirements: We will comply with all CERC guidelines for the transfer of live organisms (LOTR). Transfer Request Forms will be completed prior

to crayfish being transferred (Appendix 9). We will use a modified version of CERC Fish Transfer Treatment Procedure (Appendix 10) to treat crayfish transferred to CERC's quarantine room.

We will comply with CERC Hazard Analysis Critical Control Point (HACCP) plans for the transfer of field-collected biological, water, and sediment samples and field-deployed passive samplers (Appendix 11). We will also comply with CERC HACCP plan for field work (Appendix 11; in review). Missouri Department of Conservation will comply with their cleaning and disinfecting protocols (Appendix 12).

11. Quality Assurance Requirements: To the extent practicable, all analyses will comply with Good Laboratory Practices (GLPs). This includes descriptions of maintenance, inspections of instruments, and acceptance testing of instruments, equipment, and their components, as well as the calibration of such equipment and the maintenance of all records relating to these exercises. Documentation to be included with the final report(s) from each study will include field logs for the collection or generation of the samples, chain of custody records, and other QA/QC documentation as applicable. Requirements for analyses, sample matrices, parameters, and standard operating procedures are listed in Table 2 and 4.

12. Endpoint of Study, Based on Accomplishments: Endpoint of study will be the **c**ompletion of all chemical, biological, and statistical analyses and a peer-reviewed project completion report. Prior to submission of a publication to a scientific journal or other outlet, the USGS will provide a copy for review to the Trustee Council. USGS will provide responses to the Trustee comments on the draft publication. The Trustees will also be provided copies of the journal review comments and proposed author responses for review and comment prior to submittal of the revised manuscript to the scientific journal.

13. Schedule of Study and the Outputs Expected: Field collections will be conducted in June through August 2015, conditions permitting. Laboratory analyses will be completed by June 2016 with a draft report in review by December 2016. Annual progress reports will be provided by September 30th.

14. Place where Data will be Stored and Archived: CERC

15. Relationship to Cooperator Needs: The Department of the Interior and the State of Missouri seek to determine injury to biological resources. Crayfish play an important role in Ozark streams because of their ecological dominance (Momot 1995; Rabeni et al. 1995; Whitledge and Rabeni 1997), and because they are a primary prey of sport fishes such as smallmouth bass (*Micropterus dolomieui*), rock bass (*Ambloplites rupestris*), and longear sunfish (*Lepomis megalotis*), as well as prey for over 200 aquatic and terrestrial animals, including migratory waterfowl (Hobbs 1993; Probst et al. 1984; DiStefano 2005). The research conducted within this study plan has been specifically requested by the Missouri Department of Natural Resources and U.S Fish and Wildlife Service as a part of a National Resource Damage Assessment

and Restoration. Data will be used in various regulatory and management programs related to the effects of mining on aquatic ecosystems.

16. Literature Cited:

Allert, A.L., Fairchild, J.F., DiStefano, R.J., Schmitt C.J., Besser, J.M., Brumbaugh, W.G., and B.C. Poulton. 2008. Effects of lead-mining on crayfish (*Orconectes hylas*) in the Black River watershed, Missouri, USA. *Freshwater Crayfish* 16:97–111.

Allert, A.L., Fairchild, J.F., DiStefano, R.J., Schmitt, C.J., Brumbaugh, W.G., and J.M. Besser. 2009a. Ecological effects of lead mining on Ozark streams: In-situ toxicity to woodland crayfish (*Orconectes hylas*). *Ecotoxicology and Environmental Safety* 72(4):1207–1219.

Allert, A.L., Fairchild, J.F., Schmitt, C.J., Besser, J.M., Brumbaugh, W.G., and S.J. Olson. 2009b. Effects of mining-derived metals on riffle-dwelling benthic fishes in Southeast Missouri, USA. *Ecotoxicology and Environmental Safety* 72(6):1642–1651.

Allert, A.L., DiStefano, R.J., Schmitt, C.J., Fairchild, J.F., and W.G. Brumbaugh. 2012. Effects of mining-derived metals on riffle-dwelling crayfish in southwestern Missouri and southeastern Kansas, USA. *Archives of Environmental Contamination and Toxology* 64(4):563–572.

Allert, A.L., DiStefano, R.J., Fairchild, J.F., Schmitt, C.J., McKee, M.J., Girondo, J.A., Brumbaugh, W.G., and May T.W. 2013. Effects of historical lead–zinc mining on riffle-dwelling benthic fish and crayfish in the Big River of southeastern Missouri, USA. *Ecotoxicology* 22(3):506–521.

APHA, American Water Works Association and Water Pollution Environment Federation. 2005. Standard methods for the examination of water and wastewater. American Public Health Association, Washington, DC.

Bain, M.A., Finn, J.T., and H.E. Booke. 1985. Quantifying stream substrate for habitat analysis studies. *North American Journal of Fisheries Management* 5:499-506.

Besser, J.M., Brumbaugh, W.G. Brumbaugh, May, T.W., and C.J. Schmitt. 2007. Biomonitoring of lead, zinc, and cadmium in streams draining lead-mining and non-mining areas. *Environmental Monitoring and Assessment* 129:227–241.

Besser, J.M., Brumbaugh, W.G., Allert, A.L., Poulton, B.C., Schmitt, C.J., and C.G. Ingersoll. 2009. Ecological impacts of lead mining on Ozark streams: toxicity of sediment pore water. *Ecotoxicology and Environmental Safety* 72:516–526.

Blessing, J.J., Marshall, J.C., and S.R. Balcombe. 2010. Humane killing of fishes for scientific research: a comparison of two methods. *Journal of Fish Biology* 76:2571-2577.

Bovee, K. D. and R. Milhouse. 1978. Hydraulic simulation in instream flow studies: theory and techniques. Instream Flow Information Paper Number 5. FWS/OBS-78/33. Cooperative Instream Flow Service Group, Fort Collins, Colorado.

Brumbaugh, W.G., May, T.W., Besser, J.M., Allert, A.L., and Schmitt, C.J. 2007. Assessment of elemental concentrations in streams of the New Lead Belt in southeastern Missouri, 2002-05. U.S. Geological Survey Scientific Investigations Report 2007-5057, http://pubs.usgs.gov/sir/2007/5057/.

Charlebois, P.M and G.A. Lamberti. 1996. Invading crayfish in a Michigan stream: direct and indirect effects on periphyton and macroinvertebrates. *Journal of the North American Benthological Society* 15:(4)551–563.

DiStefano, R.J. 1993. Ecology of Stream-dwelling Crayfish Populations: A Literature Review.Missouri. Department of Conservation. 37 pps.

DiStefano, R.J., Gale, C.M., Wagner, B.A., and R.D. Zweifel. 2003. A sampling method to assess lotic crayfish communities. *Journal of Crustacean Biology* 23:678-690.

DiStefano, R.J. 2005. Trophic interactions between Missouri Ozarks stream crayfish communities and sport fish predators: increased abundance and size structure of predators cause little change in crayfish community densities. Missouri Department of Conservation. Project F-1-R-054, Study S-41, Job 4, Final Report. Columbia, Missouri.

DiStefano, R.J., Magoulick, D.D., Imhoff, E.M., and E.R. Larson. 2009. Hyporheic zone acts as refuge for imperiled crayfishes during seasonal drying of an intermittent stream. *Journal of the North American Benthological Society* 28:142–152.

Fairchild, J.F., Boyle, T.P., English, W.R., and C.F. Rabeni. 1987. Effects of sediment and contaminated sediment on structural and functional components of experimental stream ecosystems. *Water, Air, and Soil Pollution* 36: 271-293.

Freeland-Riggert, B.T. 2014. The effects of an invasive crayfish on the aquatic macroinvertebrate community in an Ozark stream. Master's Thesis. University of Central Missouri. 126pp.

Hanson, J.M., Chambers, P.A., and E.E. Prepas. 1990. Selective foraging by the crayfish *Orconectes virilis* and its impact on macroinvertebrates. *Freshwater Biology* 24:69–80.

Hobbs, H.H. 1993. Trophic relationships of North American freshwater crayfishes and shrimps. Milwaukee Public Museum, Milwaukee, Wisconsin.

Johnson, M.L., Berger, L., Philips, L., and R. Speare. 2003. Fungicidal effects of chemical disinfectants, UV light, desiccation and heat on the amphibian chytrid *Batrachochytruim dendrobatidis*. *Diseases of Aquatic Organisms* 57:255–260.

Litvan, M.E., DiStefano, R.J., Walker, K.J., and X. Gao. 2010. A recreational fishery for Longpincered Crayfish, *Orconectes longidigitus* (Faxon), in Table Rock Lake, Missouri, USA:Effects of environmental factors on trapping success. *Freshwater Crayfish* 17:91-101.

May, T.W., Wiedmeyer, R.H., Brumbaugh, W.G., and Schmitt, C.J. 1997. The determination of metals in sediment pore waters and in 1N HCl-extracted sediments by ICP-MS. *Atomic Spectroscopy,* 18**:**133-139.

Missouri Department of Natural Resources (MDNR). 1986. Preliminary assessment/site investigation report of Anschutz Mining Corporation, Fredericktown, Missouri. Missouri Department of Natural Resources, Jefferson City, Missouri.

Missouri Department of Natural Resources (MDNR). 2004. Total maximum daily load information sheet, Goose and Saline Creeks. Water Protection Program, Missouri Department of Natural Resources, Jefferson City, Missouri.

Missouri Department of Natural Resources (MDNR). 2005. Natural resources damage sampling report, Madison County Mines Tailings Superfund Site, Fredericktown, Missouri. Missouri Department of Natural Resources, Jefferson City, Missouri.

Missouri Department of Natural Resources (MDNR). 2010. Total maximum daily load information sheet, Village Creek. Water Protection Program, Missouri Department of Natural Resources, Jefferson City, Missouri.

Momot, W.T. 1995. Redefining the role of crayfish in aquatic ecosystems. *Reviews in Fisheries Science,* 3(1)**:**33-63.

Pfleiger, W.L. 1996. The crayfishes of Missouri. Missouri Department of Conservation, Jefferson City, Missouri.

Pfleiger, W.L. 1997. The fishes of Missouri. Missouri Department of Conservation, Jefferson City, Missouri.

Probst, W.E., Rabeni, C.F., Covington, W.G., and Marteney, R.E. 1984. Resource use by stream-dwelling rock bass and smallmouth bass. *Transactions of the American Fisheries Society,* 113(3)**:**283-294.

Rabeni, C.F., Gossett, M., and McClendon, D.D. 1995. Contribution of crayfish to benthic invertebrate production and trophic ecology of an Ozark stream. *Freshwater Crayfish,* 10**:**163-173.

U.S. Environmental Protection Agency (USEPA). 2006. Madison County Mine Site ecological risk assessment. Operable Unit 3 Superfund Site: CERCLIS ID#: MODO98633415-OU3. Kansas City, Kansas.

U.S. Fish and Wildlife (USFWS) and Missouri Department of Natural Resources (MDNR). 2015. Preassessment screen and determination. Madison County Mines Site, Madison County, Missouri. 25pp.

Whitledge, G.W., and Rabeni, C.F. 1997. Energy sources and ecological role of crayfishes in an Ozark stream: Insights from stable isotopes and gut analysis. *Canadian Journal of Fisheries and Aquatic Sciences,* 54(11)**:**2555-2563.

Williams, K., Brewer, S.K., and M.R. Ellersieck. 2014. A comparison of two gears for quantifying abundance of lotic-dwelling crayfish. *Journal of Crustacean Biology* 34(1):54-60.

Wilson, J.M., Bunte, R.M., and A.J. Carty. 2009. Evaluation of rapid cooling and tricaine methansulfonate (MS222) as methods of euthanasia in zebrafish (*Danio rerio*). *Journal of the American Association for Laboratory Animal Science* 48(6):785–789.

Wismer, D.A. and A.E. Christie. 1987. Temperature relationships of Great Lake fishes: a data compilation. Great Lakes Fishery Commission Special Publication No. 87-3. Ann Arbor, Michigan. 165pp.

Wooster-Brown, C. 2006. Draft Report Madison County Mine Site Ecological Risk Assessment: Ecological Risk Assessment Madison County Mine Operable Unit 3 Superfund Site CERCLIS ID# MODO98633415-OU3. U.S. Environmental Protection Agency, Region 7, Kansas City, Kansas. 80pp.

17. Signatures:

Prepared by: _____ Date: _____
Ann L. Allert
Principal Investigator

Prepared by: _____ Date: _____
William G. Brumbaugh
Research Chemist

Approved by: _____ Date: _____
Jo Ellen Hinck
Center Contact

Approved by: _____ Date: _____
Edward E. Little
Branch Chief

Approved by: _____ Date: _____
C. Ryan Warbritton
Animal Care and Use Committee Chair

Approved by: _____ Date: _____
James Candrl
Biosecurity Coordinator

Approved by: _____ Date: _____
Frank Proa
Quality Assurance and Safety Officer

Approved by: _____ Date: _____
Rip Shively
Center Director

Table 1: List of proposed study sites for population study and in-situ toxicity test and metals concentrations in dried sediments as determined by X-ray fluorescence (XRF) meter data.

Site ID	Description	Classification[a]	Abundance Estimates	In-situ Cage Study	Comments
1	Upper Little St. Francis River off Tillman Road	Low	Yes	Yes	
2	Little St. Francis River downstream of Village Creek		Yes		
3	Saline Creek at South Chamber Street (City of Fredericktown Police Training Facility)		Yes	Yes	
4	Little St. Francis River downstream of Saline Creek		Yes	Yes	
5	Little St. Francis River downstream of Madison CR 530 (downstream of the Narrows)		Yes		
6	Little St. Francis River at MDC Thompson Ford Access		Yes		
7	Chapel Creek off CR507	Low	Yes		
8	Lower Little St. Francis River off CR527		Yes	Yes	

Table 1 (continued)…

Sampling Location	Sediment size fraction	Number of readings and mean concentrations (µg/g or mg/kg DW)											
		Pb		Cd[b]		Zn		Ni		Cu		Co	
1	<2mm	n=4	45.5		<LOD[c]	n=4	47.3	n=4	17.0	n=4	20.5		<LOD
2	<2mm												
3	<2mm												
4	<2mm												
5	<2mm												
6	<2mm	n=4	383	n=4	<LOD	n=4	38.5	n=4	78.5	n=4	21.3	n=4	<LOD
7	<2mm	n=5	28.0		<LOD	n=5	20.4	n=5	16.2	n=5	12.4		<LOD
8	<2mm	n=10	110.3	n=10	8.7	n=10	23.4	n=10	43.2	n=10	16.0	n=1	33.0
1	>2mm	n=4	21.1	n=1	6.0	n=4	18.6	n=4	8.3	n=4	11.8	n=4	<LOD
2	>2mm												
3	>2mm												
4	>2mm												
5	>2mm												
6	>2mm	n=4	76.7	n=1	11	n=4	46.5	n=4	140.3	n=4	57.3	n=1	558

Sampling Location	Sediment size fraction	Number of readings and mean concentrations (µg/g or mg/kg DW)											
		Pb		Cd[b]		Zn		Ni		Cu		Co
7	>2mm	n=5	20.1	n=2	9.5	n=5	19.0	n=5	12.0	n=5	14.5	<LOD
8	>2mm	n=10	70.2		<LOD	n=10	44.8	n=10	57.5	n=10	12.8	<LOD
Probable Effects Concentrations[d] (above which harmful effects likely)			128.0		4.98		459.0		48.6		149.0	ND[e]

[a] Based pre-assessment XRF data and PEC values developed by MacDonald et al. (2000).
[b] Missouri Department of Natural Resources XRF LOD is >20 ppm for Cd.
[c] LOD = Level of Detection.
[d] MacDonald et al. (2000).
[e] Not determined.

Table 2: Proposed requirements for accuracy, precision and detection limits.

Parameter	**Estimated Accuracy for each matrix**	**Estimated Precision for each matrix**	**Precision Protocol for each matrix**	**Estimated Detection Limit**
Chemical	Measure Values within 95% of CI or 10% of Mean	Replicate Values within ± 25%	Analyze duplicate at least once per run	Temperature (±0.2°C)
				pH (±0.2 unit)
				Turbidity (±5% of reading ±1 NTU @ temperature of calibration)
				Conductivity (100 μmhos/cm)
				Dissolved oxygen (±0.2 mg/L ≤ 20 mg/L)
				Metals (varies)
				Nutrients (varies)
				Dissolved and particulate organic carbon (20 μg/L)
				Chlorophyll *a* (0.5 μg/L)
				Cations/anions (varies)
				Total suspended solids (1 mg/L)
				Alkalinity and hardness (2 mg/L)
				Total organic carbon (sediment) (20 μg/L)
Habitat				GPS (10 m)
				Stream order, watershed area, mining-related materials (varies)

Table 3: Proposed quality assurance samples for various matrices.

Type	Matrix	Frequency	Analysis	Rationale
Field Duplicates	Water	1 per run	YSI or Hydrolab®, water quality multi-parameter meter, trace metals	Measures precision of sample collection and degree of environmental variability
Filtration Blanks	DI water	1 per field samples	Metals, water quality parameters	Monitors procedural contamination
Analytical duplicate or triplicate	Crayfish, Water, Detritus	1 per 20 analyses	Metals, water quality parameters, PSA, carbon analyses	Monitors instrumental precision
Analytical Spike	Crayfish, Water, Detritus	1 per analytical run per matrix	Metals	Monitors instrumental accuracy
Laboratory Control Sample	Crayfish, Water, Detritus, Sediment	2 per analytical run	Metals, water quality parameters, carbon analyses	Monitors instrumental accuracy
Laboratory Control Sample	Crayfish	All Voucher specimens	Identification	Monitors technician accuracy
Calibration Standard	Crayfish, Water, Detritus, Sediment	1 per analytical run	Metals, YSI or Hydrolab® water quality paramenters, carbon analyses	Monitors accuracy

Table 4: Proposed sample matrices, parameters and analytical methods or standard operating procedures (SOPs).

Matrix	**Parameter**	**Analytical Methods**
General Laboratory Practices		B4.01, B4.44, B5.03, B5.16, B5.40, B5.63, B5.106, APHA 2005
Water	Temperature	SOP B5.6, APHA 2005, instrument manuals
Water	pH	SOPs B4.14; B4.56, B4.62, B5.239, APHA 2005, instrument manuals
Water	Conductivity	Proposed, APHA 2005, instrument manuals
Water	Dissolved oxygen	Proposed, APHA 2005, instrument manuals
Water	Turbidity	SOP B4.42, APHA 2005, instrument manuals
Water	Total suspended solids	SOP F6.2.15.022696
Water	Alkalinity	SOP B4.16, APHA 2005
Water	Hardness	SOP B5.95, APHA 2005
Water	Anions	SOP P.705
Water	Cations	SOP P.241
Water	Nutrients	F6.2.22, APHA 2005, instrument manuals
Water	Dissolved organic carbon, chlorophyll *a*	SOP P.722, B5.37, APHA 2005, instrument manuals
Crayfish	Animal care	B5.72.091997, B5.13.052693, P.691 (B5.148.030789), P.683 (B5.154.091997), P.690 (B5.160.013189), B5.165.091997, B5.240.060392, F5-15-020488, P.691
Sediment	Carbon	SOP B4.36, F.6.20.1.082396, APHA 2005, instrument manuals
Sediment	Particle size	B5.179, APHA 2005
Metals	Crayfish, detritus, sediment, water	SOPs C5.5, P.485, P.259, P.221, P.510, P.636, P.198, P.241, P.213, P.184, P.239
Habitat variables	Velocity, depth, in-situ substrate quality	See attached protocols

Types of quality control for quantitative analysis by ICP-MS are indicated in SOPs C5.135, C5.212. Corrective actions are specified in SOP C5.209. Procedures for calculating QC statistics are as follows:

Percent Relative Standard Deviation (%RSD) = SD/Mean x 100
Relative Percent Difference or RPD = (D1-D2)/Mean x 100
% Spike Recovery = (Total Measured – Background)/Spike Amount x 100
Method Limit of Detection = $3 \times (SD_b^2 + SD_s^2)^{1/2}$ where

SD_b = standard deviation of a blank or low level standard and
Sd_s = standard deviation of a low level sample.

Table 5: Proposed water quality, sediment, and biotic variables to be measured.

Matrix	Variable	No. Reps / Site	Where measured
Surface	Temperature	3	In situ
Surface	pH	3	In situ
Surface/ pore water	Conductivity	3	In situ
Surface	Dissolved Oxygen	3	In situ
Surface	Turbidity	3	In situ
Surface/ pore water	Alkalinity	3 / 1	Lab
Surface/ pore water	Hardness	3 / 1	Lab
Surface/ pore water	DOC	3 / 1	Lab
Surface	Anions, cations	3	Lab
Surface/ pore water	Selected metals	3 / 1	Lab
Surface	Nutrients (NH_3, TN, TP, TSS, POC, chl *a*)	3	Lab
Crayfish	Density	21 kicks	In situ
Crayfish	Mortality	3 cages (n =10) per date per species	In situ
Population study: crayfish	Selected metals	3 composited samples per date	Lab
Cage study: crayfish	Selected metals	3 composites (max. n =10) per date per species	Lab
Cage study: leaves, invertebrates, fish	Selected metals	3	Lab

Table 6: Proposed habitat and sediment quality variables to be measured.

Matrix	Variable	No. Reps / Site	Where measured
Surface water	Current velocity	minimum of 7 per riffle/ 21 per site	In situ
Surface water	Depth	minimum of 7 per riffle / 21 per site	In situ
Sediment	Sediment particle size characterization	minimum of 7 per riffle; 21 per site	In situ
Sediment	Sediment carbon	3	Lab
Sediment	Selected metals	6 (3 per fraction)	Lab
Site	Stream order	1	Lab
Site	Coordinates	1–4 (each riffle)	In situ
Site	Watershed area	1	Lab
Site	Land use	1	Lab
Site	Stream discharge	1	In situ

Table 7: Proposed project budget to conduct a survey of crayfish populations and in-situ toxicity study using endemic crayfish in the Little St. Francis River watershed in Madison County, Missouri, USA.

Study	**Cost**	**Comments**
Population study	$160,775.00	
Analytical support for population study	$33,000.00	
In-situ cage study	$56,380.00	
Analytical support for cage study	$40,500.00	
Sub-total	**$349,905.00**	
Overhead	**$24,493.35**	
Total	**$374,398.35**	
	In-Kind Contributions	
Population, cage, & analytical	*$468,550.00*	
MDC	*$37,500.00*	
	$506,050.00	

Appendices

Apprendix 1: Datasheets

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

Chain-of-Custody Record

Attachment 1

Study No.	Study Name	Control No.
Samplers: (Signatures)		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 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

DRAFT

USGS
science for a changing world

United States Department of the Interior

II

U.S.GEOLOGICAL SURVEY
Columbia Environmental Research Center
4200 New Haven Road
Columbia, Missouri 65201

SAMPLE BATCH HISTORY INFORMATION
FOR SAMPLES RECEIVED AT ECRC

PROJECT LEADER, STUDY DIRECTOR,
OR BIOMONITORING SPECIALIST: _____

FY: _____ PROJECT OR STUDY #: _____ WU#: _____

DATE: _____ OTHER INFORMATION: _____

BRIEF DESCRIPTION OF BATCH: _____

_____

_____

TOTAL NUMBER OF SAMPLES IN BATCH: _____

TYPE(S) OF SAMPLES IN BATCH: _____

_____

COLLECTED BY: _____

DATE(S) COLLECTED: _____

COLLECTION SOP'S : _____, _____

GENERAL GEOGRAPHICAL AREA(S) OF COLLECTION: _____

_____

HOW ARE SAMPLES PACKAGED? _____

_____

_____

WHO PACKAGED SAMPLES? _____

METHOD(S) OF PRESERVATION: _____

_____

_____

NUMBER OF COOLERS, BOXES, ETC. COMPRISING BATCH: _____

WERE SAMPLES STORED BEFORE TRANSMISSION? _____

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

_____

SAMPLE BATCH HISTORY INFORMATION (continued)

_____

DRAFT

TRANSMISSION DATE:____/____/____

TRANSMITTED FROM:_____

TRANSMITTED TO:_____

MODE OF TRANSMISSION (HOW ARE YOU SENDING THE SAMPLES?):

_____

ANALYSES REQUESTED:_____

_____

_____

DESCRIBE ANY DOCUMENTS ACCOMPANYING THE SAMPLES:_____

_____

_____

ADDITIONAL COMMENTS:_____

_____

_____

_____

SIGNATURE OF INDIVIDUAL ULTIMATELY RESPONSIBLE FOR THE SAMPLES:

NAME:_____

ADDRESS:_____

_____

_____

PHONE: ( )_____ - _____

FAX: ( )_____ - _____

DRAFT

Madison Co. Mines 2015 - MDC Crayfish Sampling Page:___of___

Stream:	Site:	Date:	
Water Temp:	Personnel:	Gear Type:	Time:
Sample Number:	UTM Coordinates:		
Habitat Description: 1-Riffle 2-Run 3-Pool 4-Veg 5-Back Wat Rabeni Number = _____ %Veg Cover_____	Depth (m):	C.V. (m/s):	
Substrate: %Boulder_____ %Cobble_____ %Pebble_____ %Gravel_____ %Sand_____ %Detritus_____			SiltCov= L M H

C. diogenes=**DI**, C. hubbsi=**HU**, C. maculatus=**MA**, O. harrisoni=**HA**, O. hylas=**HY**, O. immunis=**IM**, O. longidigitus= **LO**, O. luteus=**LU**, O. medius=**MD**, O. meeki=**MK**, O. neglectus=**NE**, O. ozarkae=**OZ**, O. peruncus=**PE**, O. punctimanus=**PU**, O. quadruncus=**QU**, O. virilis=**VI**, O. williamsi=**WI**, P. acutus=**AC**, P. clarkii=**CL**, P. gracilis=**GR**, Unknown=**UK**, Note Codes: Buster=B, Dead=D, Recent Molt=R, w/eggs=E, w/hatchlings=X, Genetic Sample=G

Crayfish #	Species	Sex	Form	CL(mm)	CW(mm)	WT(g)	Notes

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MDC Crayfish Codes

Number	Species	New Codes	Old Codes(pre2002)		
1	Cambarellus puer	PR			
2	Cambarellus shufeldtii	SH			
3	Cambarus diogenes	DI			
4	Cambarus hubbsi	HU	H		
5	Cambarus hubrichti	HB			
6	Cambarus maculatus	MA	CM		
7	Cambarus setosus	SE			
8	Fallicambarus fodiens	FO			
9	Faxonella clypeata	CY			
10	Orconectes eupunctus	EU			
11	Orconectes harrisoni	HA	OH		
12	Orconectes hylas	HY	Y		
13	Orconectes immunis	IM			
14	Orconectes lancifer	LA			
15	Orconectes longidigitus	LO	G		
16	Orconectes luteus	LU	L		
17	Orconectes marcus	MR			
18	Orconectes marchandi	MC			
19	Orconectes medius	MD	OM		
20	Orconectes meeki	MK	M		
21	Orconectes neglectus	NE	N		
22	Orconectes ozarkae	OZ	O		
23	Orconectes palmeri	PA			
24	Orconectes peruncus	PE	E		
25	Orconectes punctimanus	PU	P		
26	Orconectes quadruncus	QU	Q		
27	Orconectes virilis	VI	V		
28	Orconectes williamsi	WI	W		
29	Procambarus acutus	AC			
30	Procambarus clarki	CL			
31	Procambarus gracilis	GR			
32	Procambarus viaeveridus	VV			
33					

Largemouth Bass = L, Smallmouth Bass = S, Rock Bass = R, Green Sunfish = G, Longear Sunfish = E, Bluegill = B
Yellow Bullhead = Y, Flathead = F, Sculpin = C, Madtom = M.

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Page ___ of ___

Madison Co. Mines

Ammonia data

Site	Site description	Riffle	Rep	Collection date	Collection time	Analysis date	NH_3 (ug/L)	NH_3 temp	Comment
						Tech:			
						Meter:			

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Run standards for each analysis

Madison County Mines (MCM) Bench Water Quality

Site	Site Descrip.	Riffle #	Date of Collection	Analysis date	Time of Analysis	Lab Temp C	Lab pH	Sample vol. (ml)	mls titrant added	Lab alk (mg/L)	mls titrant added	Lab Hard (mg/L)	Turb (NTU)	Comments
QA	Turb Stnd (conc =)													
QA	CERC Well Water													
QA	Alk Stnd (conc =)													
QA	Hard Stnd (conc =)													
		meter												
		analyst												

Record all conc and all reps for Alk, Hard, Turb Stnds

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Madison Bench XRF Data

					Element (ppm)												
Site No.	Site Descrip.	Analysis Date	Time of Analysis	Shot # of trip	Co	±2δ	Pb	±2δ	Zn	±2δ	Cd	±2δ	Ni	±2δ	Cu	±2δ	Comments

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Madison Co. Mines 2015 Cage Crayfish Length Weight Page:___of___

Site: Riffle: Date:

Cage No.	Crayfish #	Species	Sex	Form	CL(mm)	Wt (g)	Notes

DRAFT

Madison Co. Mines 2015

Cage Habitat	**Date:**	**Techs:**	**Page ____ of ____**
Site No.:	**Site Name:**		

	Cage Number											
Parameter												
Substrate												
Substrate												
Substrate												
Substrate												
Substrate												
Depth (cm)												
Vel. At 2 cm(m/s)												
Vel. At 0.6 (m/s)												

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Madison Co. Mines 2015
Crayfish Stocking Sheet

Site: _____ Date: _____ Initials: _____

Cage No.	Species	Time	Temp in Container (°C)	Temp in Stream (°C)	Number of Crayfish	Feed?	Portal Closd?	Comments

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Chlorophyll Data Form

Ecology Section

Study:						**No.**		
Date of analysis:				**Analyst Signature:**				
Meter mfg./ model no.:			**Date of calibration:**				**Calibration factor:**	
CERC #	**Sample ID**	**Sample collection date**	**Volume water filtered (ml)**	**Volume acetone extract (ml)**	**Reading before acid (µg/L)**	**Reading after acid (µg/L)**	**Dilution factor**	**Comments**

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Madison Co. Mines 2015	Page:___of___
Crayfish TIME ZERO	Date:

Crayfish will be measured from those transported to the field for stocking. Although these crayfish are generally smaller than those stocked, we will be able to develop a length-weight relationship for each species with this data. Please indicate individuals from coolers with a "C". THESE SHOULD NOT BE SUBMITTED FOR METAL SAMPLES.

Crayfish from the diluters or waterbaths will be collected for metal samples. Please indicate individuals from waterbath/tank with a "W". We would like 3 reps from each species, if possible.

Crayfish #	Species	Cooler / waterbath	Sex	Form	CL(mm)	WT(g)	Notes

DRAFT

Cross-Sectional Depth and Velocity Measurements

Study: Madison Co. Mines 2015	Site Number:
Date:	Technicians:

Page ____ of ____

	Interval b/w transects (m):				Measurements (**looking upstream, left to right**)									
Distance between stations (m)	Riffle # / transect #	Distance from Bottom of riffle (m)	Wetted Bank Width (m)	Parameter	1	2	3	4	5	6	7	8	9	10
				Depth (cm)										
				Vel. At 0.6 (m/s)										
				Vel. At 2 cm(m/s)										
				Depth (cm)										
				Vel. At 0.6 (m/s)										
				Vel. At 2 cm(m/s)										
				Depth (cm)										
				Vel. At 0.6 (m/s)										
				Vel. At 2 cm(m/s)										
				Depth (cm)										
				Vel. At 0.6 (m/s)										
				Vel. At 2 cm(m/s)										
				Depth (cm)										
				Vel. At 0.6 (m/s)										
				Vel. At 2 cm(m/s)										

Start numbering at furtherest downstream riffle, i.e., upstr riffle, three transect = 3/3)

DRAFT

Cross-Sectional Substrate Measurements

Study: Madison Co. Mines 2015	Site No.:	Date:	Techs:	Page ____ of ____
Interval b/w transects (m):		Measurements (**looking upstream, left to right**)		

Distance between stations (m)	Riffle # / transect #	Distance from Bottom of riffle (m)	Wetted Bank Width (m)	Parameter	1	2	3	4	5	6	7	8	9	10
				Substrate										
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				Substrate										

Start numbering at furtherest downstream riffle, i.e., upstr riffle, three transect = 3/3)

Substrate classes: 1 - silt/sand; 1 = bedrock; 2 = gravel; 3 = pebble; 4 = cobble; 5 = boulder; 6 = irregular bedrock

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Detritus Sampling Dataform – Madison Project 2015

Site	Riffle No.	Date / Time	Comments (Percentage of Different Materials taken for Metal Sample; Percentage from each cage)
			Initials
			Initials
			Initials

Start numbering at downstream riffle (most downstream riffle = 1; most upstream riffle = 3)

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MC2015 Field Water Quality Datasheet

Page ____ of ____

Technicians: **Meter:** **Date:**

Site #	Site Name	Riffle (1, 2 or 3)	Equip. Used	Time	Water Temp (°C)	pH	Cond. (µmhos)	DO (mg/L)	Turb (NTU)	Comments
pH stnd (conc =)										
cond stnd (conc =)										
DO (temp =) (BPmmHg =) (Chart mg/L =) (Actual mg/L =)										
Record all stnds for pH, cond										

DRAFT

GPS Dataform

Page __ of ___

Study Name: Madison 2015

Technicians:

		North			West						Comments
Site	Riffle	Deg	Min	Sec	Deg	Min	Sec	Date	Time	Initials	

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Meter:
Meter S/N:

Madison Co. Mines 2015 **POC Sample Preparation Datasheet**

Date Collected:		Date Filtered:	Techs:
Time:		Time:	Filter Lot #:
Date Acidified:			

Site ID	pH	Temp	POC volume filtered	Comments

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Madison Co. Mines 2015

Particulate Carbon Analysis

Sample batch ID :

Meter:

Sample collection date(s) :

Run date : **Technician :**

	Sample ID	Standard ID	Sample volume (L)	Coulometer output (ug C)	Comments
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					

notes:

DRAFT

Date: _____ Crew: _____

Deployment Checklist - Madison Co. Mines 2015

Site	Date	Cage No.s	Door closure	Tether	Current velocity	Substrate charac.	Peeper Deployment	In-Situ WQ	TempPro Deployment	Sign Posted?	Comments

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Madison Co. Mines 2015 Sample Processing Check-off Datasheet for Survey

Page ____ of ____

Note: all samples processed from 1-gal surface grab sample or sediment sample collected at each riffle from each site
Most downstream riffle = 1; most upstream riffle = 3

Signatures:					*(Initial below to indicate done)*								
Site No./ Riffle No.	**Alk / hard / turb)** (1gal, ice)	**TN/TP** (60 ml, ice)	**Ammonia** (60 ml, filtered, ice)	**Anions** (15ml, filtered, ice)	**Metals and cations** (20 ml filtered, ice)	**DOC** (20-ml filtered, ice)	**Chl *a*** (100 ml, place filter in foil, ice)	**POC** (250-500 ml filtered in foil, ice)	**TSS** (500-1 L, filter, dried)	**Sediment Metals** (<2 mm, ice)	**Sediment** (<250 µm, @CERC sieve, refrig)	**TOC sediment** (@CERC split from <2 mm + <250 µm, refrig)	**Crayfish** (125 ml, ice)

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Site Characterization Dataform

Madison Co. Mines 2015	**Page __ of ___**
Technicians:	
Site-Station:	**Date:**

Bank Condition:

Comments:

Map:

Start numbering at downstream riffle (most downstream riffle = 1; most upstream riffle = 3)

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Madison Co. Mines 2015

TempPro Data

Site	TempPro Numbers	Date/Time Deployed	Light Flashing?	Date/Time Retreived	Where Deployed?

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TOTAL SUSPENDED SOLIDS WORKSHEET

Madison Co. Mines 2015

Sample collection dates:

Dates of analysis: **Analyst:**

Filter type: **Lot no.:**

Standard cat no.: **Standard lot no.:** **Standard value:**

Oven: **Temp (°C):**

Drying time First : _____ hrs Second: _____ hrs.

Sample ID	Sub-sample desig.	Filter no.	Filter wt. (g)	Vol. filtered (ml)	Filter & residue mass (g)	Re-weigh filter & residue mass (g)	Comments

Balance:
Calibration:

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Qualitative Habitat Evaluation Index and Use Assessment Field Sheet

OhioEPA

QHEI Score: ☐

Stream & Location: _____ **RM:** _ _ _._ **Date:** _ _/ _ _/ 06

Scorers Full Name & Affiliation: _____

River Code: _ _ _- _ _ _- _ _ _ **STORET #:** _ _ _ _ _ _ **Lat./ Long.:** (NAD 83 - decimal °) _ _ . _ _ _ _ /8_ . _ _ _ _ Office verified location ☐

1] SUBSTRATE

Check ONLY Two substrate TYPE BOXES; estimate % or note every type present

BEST TYPES	POOL	RIFFLE	OTHER TYPES	POOL	RIFFLE
☐ ☐ BLDR /SLABS [10]	____	____	☐ ☐ HARDPAN [4]	____	____
☐ ☐ BOULDER [9]	____	____	☐ ☐ DETRITUS [3]	____	____
☐ ☐ COBBLE [8]	____	____	☐ ☐ MUCK [2]	____	____
☐ ☐ GRAVEL [7]	____	____	☐ ☐ SILT [2]	____	____
☐ ☐ SAND [6]	____	____	☐ ☐ ARTIFICIAL [0]	____	____
☐ ☐ BEDROCK [5]	____	____	(Score natural substrates; ignore sludge from point-sources)		

NUMBER OF BEST TYPES: ☐ 4 or more [2] ☐ 3 or less [0]

Check ONE (Or 2 & average)

ORIGIN	QUALITY
☐ LIMESTONE [1]	☐ HEAVY [-2]
☐ TILLS [1]	☐ MODERATE [-1]
☐ WETLANDS [0]	☐ NORMAL [0]
☐ HARDPAN [0]	☐ FREE [1]
☐ SANDSTONE [0]	☐ EXTENSIVE [-2]
☐ RIP/RAP [0]	☐ MODERATE [-1]
☐ LACUSTURINE [0]	☐ NORMAL [0]
☐ SHALE [-1]	☐ NONE [1]
☐ COAL FINES [-2]	

SILT

EMBEDDEDNESS

Comments

Substrate ☐ Maximum 20

2] INSTREAM COVER

Indicate presence 0 to 3: 0-Absent; 1-Very small amounts or if more common of marginal quality; 2-Moderate amounts, but not of highest quality or in small amounts of highest quality; 3-Highest quality in moderate or greater amounts (e.g., very large boulders in deep or fast water, large diameter log that is stable, well developed rootwad in deep / fast water, or deep, well-defined, functional pools.

____ UNDERCUT BANKS [1]	____ POOLS > 70cm [2]	____ OXBOWS, BACKWATERS [1]
____ OVERHANGING VEGETATION [1]	____ ROOTWADS [1]	____ AQUATIC MACROPHYTES [1]
____ SHALLOWS (IN SLOW WATER) [1]	____ BOULDERS [1]	____ LOGS OR WOODY DEBRIS [1]
____ ROOTMATS [1]		

AMOUNT

Check ONE (Or 2 & average)

☐ EXTENSIVE >75% [11]
☐ MODERATE 25-75% [7]
☐ SPARSE 5-<25% [3]
☐ NEARLY ABSENT <5% [1]

Comments

Cover ☐ Maximum 20

3] CHANNEL MORPHOLOGY

Check ONE in each category (Or 2 & average)

SINUOSITY	DEVELOPMENT	CHANNELIZATION	STABILITY
☐ HIGH [4]	☐ EXCELLENT [7]	☐ NONE [6]	☐ HIGH [3]
☐ MODERATE [3]	☐ GOOD [5]	☐ RECOVERED [4]	☐ MODERATE [2]
☐ LOW [2]	☐ FAIR [3]	☐ RECOVERING [3]	☐ LOW [1]
☐ NONE [1]	☐ POOR [1]	☐ RECENT OR NO RECOVERY [1]	

Comments

Channel ☐ Maximum 20

4] BANK EROSION AND RIPARIAN ZONE

Check ONE in each category for EACH BANK (Or 2 per bank & average)

River right looking downstream

EROSION (L R)	RIPARIAN WIDTH (L R)	FLOOD PLAIN QUALITY (L R)	(L R)
☐ ☐ NONE / LITTLE [3]	☐ ☐ WIDE > 50m [4]	☐ ☐ FOREST, SWAMP [3]	☐ ☐ CONSERVATION TILLAGE [1]
☐ ☐ MODERATE [2]	☐ ☐ MODERATE 10-50m [3]	☐ ☐ SHRUB OR OLD FIELD [2]	☐ ☐ URBAN OR INDUSTRIAL [0]
☐ ☐ HEAVY / SEVERE [1]	☐ ☐ NARROW 5-10m [2]	☐ ☐ RESIDENTIAL, PARK, NEW FIELD [1]	☐ ☐ MINING / CONSTRUCTION [0]
	☐ ☐ VERY NARROW < 5m [1]	☐ ☐ FENCED PASTURE [1]	
	☐ ☐ NONE [0]	☐ ☐ OPEN PASTURE, ROWCROP [0]	

Indicate predominant land use(s) past 100m riparian.

Comments

Riparian ☐ Maximum 10

5] POOL / GLIDE AND RIFFLE / RUN QUALITY

MAXIMUM DEPTH — Check ONE (ONLY!)

☐ > 1m [6]
☐ 0.7-<1m [4]
☐ 0.4-<0.7m [2]
☐ 0.2-<0.4m [1]
☐ < 0.2m [0]

CHANNEL WIDTH — Check ONE (Or 2 & average)

☐ POOL WIDTH > RIFFLE WIDTH [2]
☐ POOL WIDTH = RIFFLE WIDTH [1]
☐ POOL WIDTH > RIFFLE WIDTH [0]

CURRENT VELOCITY — Check ALL that apply

☐ TORRENTIAL [-1] ☐ SLOW [1]
☐ VERY FAST [1] ☐ INTERSTITIAL [-1]
☐ FAST [1] ☐ INTERMITTENT [-2]
☐ MODERATE [1] ☐ EDDIES [1]

Indicate for reach - pools and riffles.

Recreation Potential
Primary Contact
Secondary Contact
(circle one and comment on back)

Comments

Pool / Current ☐ Maximum 12

Indicate for functional riffles; Best areas must be large enough to support a population of riffle-obligate species: Check ONE (Or 2 & average). ☐ NO RIFFLE [metric=0]

RIFFLE DEPTH	RUN DEPTH	RIFFLE / RUN SUBSTRATE	RIFFLE / RUN EMBEDDEDNESS
☐ BEST AREAS > 10cm [2]	☐ MAXIMUM > 50cm [2]	☐ STABLE (e.g., Cobble, Boulder) [2]	☐ NONE [2]
☐ BEST AREAS 5-10cm [1]	☐ MAXIMUM < 50cm [1]	☐ MOD. STABLE (e.g., Large Gravel) [1]	☐ LOW [1]
☐ BEST AREAS < 5cm [metric=0]		☐ UNSTABLE (e.g., Fine Gravel, Sand) [0]	☐ MODERATE [0]
			☐ EXTENSIVE [-1]

Comments

Riffle / Run ☐ Maximum 8

6] GRADIENT

(ft/mi) ☐ VERY LOW - LOW [2-4]
☐ MODERATE [6-10]
☐ HIGH - VERY HIGH [10-6]

DRAINAGE AREA (mi²)

%POOL: ☐ %GLIDE: ☐
%RUN: ☐ %RIFFLE: ☐

Gradient ☐ Maximum 10

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Madison County Brood Stock Record				Spring 2015				
Month/year:			Species:					
Day of Month	Tank	Aeration	Water Flow	Water T° C	Feed	Siphon	Comments	Initials
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
16								
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22								
23								
24								
25								
26								
27								
28								
29								
30								
31								

DRAFT

Madison Co. Mines Assessment
Foil Pack Drying Record

Batch description	Oven mfg./model	Oven temp (°C)	Date in	Time in	Date out	Time out	Date in	Time in	Date out	Time out	Tech

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Madison County Mines

Foil Pack Weight

	Balance			Balance			
	Calib.			Calib.			
	Initial weight			Re-dried wt.			
Foil pack no.	Date	Foil pack wt. (g)	Tech.	Date	Foil pack wt. (g)	Tech.	Comments

DRAFT

Madison Co. Mines Assessment
Leaf Pack Drying Record

		Initial drying								Re-drying							
		In				Out				In				Out			
Batch description	Oven mfg./model	Date	Time	Temp (°C)	Tech	Date	Time	Temp (°C)	Tech	Date	Time	Temp (°C)	Tech	Date	Time	Temp (°C)	Tech

DRAFT

Madison County Mines

Leaf pack stocking and weight

Initial stocking: 2 Sycamore, 4 Cottonwood, 1 White oak, 1 Shagbark hickory, 15 Black willow

Leaf stocking tech.:

	Balance					**Balance**			**Balance**			
	Calib.					**Calib.**			**Calib.**			
						Initial dry weight			**Re-dried wt.**			
Date stocked	**Foil pack no.**	**# BW (if not 15)**	**# CW (if not 4)**	**Pre-dried wt. (g)**	**Tech**	**Date**	**Foil pack wt. (g)**	**Tech.**	**Date**	**Foil pack wt. (g)**	**Tech.**	**Comments**

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Appendix 2: Crayfish, Fish, and Detritus Metals Samples

Objective: Environmental samples will be taken and analyzed for metals to determine impacts of mining on crayfish populations and caged crayfish.

Crayfish: After crayfish are collected from quadrat samples, identified, sex determined and measured, they should be placed in 4-oz. pre-cleaned polypropylene (PP) jars. Three to five crayfish of the same species from each riffle at each site should be placed in separate 4-oz. PP pre-labeled jar. If only one riffle is sampled, three independent samples should be taken from that riffle. Crayfish used for metals analyses **will be identified on the data sheet**. Jars should be placed on ice until they can be placed in a freezer at hotel or CERC.

After crayfish are collected from cages, identified, sex determined, CL measured, and weighed, they should be placed in 4-oz. pre-cleaned polypropylene (PP) jars. All crayfish from a cage at each site should be placed in separate 4-oz. PP pre-labeled jar **(i.e., for a site, there should be three jars)**. Crayfish used for metals analyses **will be identified on the data sheet**. Jars should be placed on ice until they can be placed in a freezer at hotel or CERC.

Data to be taken: Total number of crayfish; carapace length (mm) of crayfish; metals (Pb, Cd, Zn, Ni, Co, Cu) in whole crayfish.

Fish: After fish are collected from kick seines or electrofishing, identified, and measured, they should be placed in 4-oz. pre-cleaned polypropylene (PP) jars. Jars should be placed on ice until they can be placed in a freezer at hotel or CERC.

Data to be taken: Total number of fish; total length (mm); metals (Pb, Cd, Zn, Ni, Co, Cu) in whole fish.

Detritus: After leaves are collected from each cage, they should be placed in 4-oz. pre-cleaned polypropylene (PP) jars. Jars should be placed on ice until they can be placed in a freezer at hotel or CERC.

Data to be taken: Weight loss, metals (Pb, Cd, Zn, Ni, Co, Cu) in detritus.

Appendix 3: Surface Substrate Composition, Current Velocity, and Depth at Riffles

Objectives: To characterize microhabitats of riffles. Data will be used to determine whether surface substrate composition, current velocity, and depth help explain densities of crayfish, and whether the kick seine locations within riffles were representative of the riffle.

Data to be recorded: Site name; site number; lateral distance between measurements for each transect (e.g., measurements obtained at left and right wetted margin and at points along transects); distance of entire riffle (e.g., downstream to upstream distance or longitudinal length); GPS coordinates for each riffle (taken at downstream end of riffle); and surface substrate size, current velocity; and depth at points along transects in each riffle. Note on data sheet position of transect station from left to right when looking either upstream or downstream.

Methods: Transects will be set up across each riffle, and measurements will be taken along each transect (see below). Distance between transects and within transects will be determined by the riffle length and width. Start at the downstream end of Riffle 1 (the furthest downstream riffle at each site). Mark each transect with numbers, starting with "1" at the most downstream end of each riffle (i.e., renumber in each riffle).

Distance between stations on each transect:
Measure wetted width of stream.
If width is <5 m, take velocity/depth measurements at 1-m intervals.
If width is 5< x <10 m, take velocity/depth measurement at 2-m intervals.
If width is 10< x <15 m, take velocity/depth measurements at 3-m intervals.
If width is 15< x <20 m, take velocity/depth measurements at 4-m intervals.

Distance to next transect:
If riffle length is <= 50 m; place next interval 10 m upstream.
If riffle length is 50< x <100 m; place next interval 20 m upstream.
If riffle length is >100 m; place next interval 30 m upstream.

Velocity Measurements:
For water depths <75 cm, measure velocity once at 0.6 of the depth from the water surface (e.g., if water is 50 cm deep, measure velocity at 30 cm from the water surface; 40 cm from the bottom surface). For water depths >75 cm, measure velocity twice at 0.2 d and 0.8 of the depth. Average these two readings to determine the velocity for that cross section. Velocity will also be measured at 2 cm above the substrate surface. Record velocity in m/sec; depth in cm.

Depth measurements:
Water depth will be measured using a standard depth gauge. Record depth in cm.

Surface substrate composition measurements:
A grid (e.g., a piece of rebar welded into an ‘X’ with each length measuring 0.5 m) will be used to characterize substrate at each point along each transect (Litvan et al. 2010). The five-pointed grid will be haphazardly dropped down on the substrate at the point where depth and velocity readings were taken. Substrate will be classified at each of the four ends of the grid (or "X") as well as the center point (5 points in total), using the following categories (from a modified Wentworth scale; Bovee and Milhouse 1978):

Sand/silt (<2 mm diameter), gravel (2 mm to 16 mm diameter), pebble (17 mm to 64 mm diameter), cobble (65 mm to 250 mm diameter), boulder (> 256 mm diameter), flat bedrock and irregular bedrock.

Each of those categories is assigned a numerical value (Bain et al. 1985):

Sand/silt = 1.0, bedrock = 1.0, gravel = 2.0, pebble = 3.0, cobble = 4.0, boulder = 5.0, irregular bedrock = 6.0

The five numerical values (from each of the five grid contact points) are recorded and averaged to obtain a mean substrate value (to the tenths decimal place) for that location along each transect.

Appendix 4: Surface Substrate Composition, Current Velocity and Depth at Quadrat Sample Locations

Objectives: To characterize microhabitat at quadrat sample locations. Data will be used to determine whether surface substrate composition, current velocity, and depth help explain densities of crayfish, and whether the quadrat sample locations within riffles were representative of the riffle.

Data to be recorded: Site name; site number; riffle number; quadrat number; substrate size class; current velocity; and depth at location of each quadrat sample.

Methods: Quadrat samples will be taken in three riffles. Placement will be determined randomly. Quadrat samples will be numbered by site number-riffle-number of quadrat with riffle (e.g., 1-1-5; 1-2-4; 1-3-3). Quadrat samples within riffles will be numbered in the order of which they are taken.

Velocity Measurements: Velocity measurements will be taken immediately adjacent to the quadrat sampler because samples taken inside the quadrat would be affected by the actual sampler. Velocity will be measured at 0.6 of the depth from the water surface (e.g., if water is 50 cm deep, measure velocity at 30 cm from the water surface; 40 cm from the bottom surface), and at 2 cm above the substrate surface. Record velocity in m/sec.

Depth measurements: Water depth will be measured in the middle of the 1-m^2 quadrat sample using a standard depth guage, and the spot where the velocity measurement is taken. Record depth in cm.

Surface substrate composition measurements: A grid (e.g., a piece of rebar welded into an 'X') will be used to characterize substrate at each quadrat sample (Litvan et al. 2010). The five-pointed grid will be haphazardly dropped down on the substrate inside the square-meter sample. Substrate will be classified at each of the four ends of the grid (or "X") as well as the center point (5 points in total), using the following categories (from a modified Wentworth scale; Bovee and Milhouse 1978):

Sand/silt (<2 mm diameter), gravel (2 mm to 16 mm diameter), pebble (17 mm to 64 mm diameter), cobble (65 mm to 250 mm diameter), boulder (> 256 mm diameter), flat bedrock and irregular bedrock.

Each of those categories is assigned a numerical value (Bain et al. 1985):
Sand/silt = 1.0, bedrock = 1.0, gravel = 2.0, pebble = 3.0, cobble = 4.0, boulder = 5.0, irregular bedrock = 6.0

The five numerical values (from each of the five grid contact points) are recorded and averaged to obtain a mean substrate value (to the tenths decimal place) for that particular quadrat sample.

Appendix 5: Processing for Water Quality Analyses

Objective: To characterized water quality in surface samples.

Methods: In-situ measurements will be taken in each riffle for temperature, pH, conductivity, turbidity and dissolved oxygen. A grab sample will also be taken in each riffle. Samples should be taken at the upstream end of the riffle.

A 1-gal (3.785 L) pre-cleaned LDPE bottle will be used to collect three grab samples per site. Grab samples should be taken starting downstream and moving upstream. Bottles should be rinsed once with site water prior to the sampling being taken. Bottle should be placed completely under the water surface and filled. Cap bottle underwater, to insure the bottle is as full as possible. Place bottle in cooler with ice.

Data to be taken: Temperature, pH, conductivity, dissolved oxygen, alkalinity, hardness, turbidity, total phosphorous (TP), total nitrogen (TN), ammonia, particulate organic carbon (POC), and total suspended solids (TSS), chlorophyll *a*.

Equipment needed: Coolers with blue ice; Hydrolab Quanta or equivalent water quality instruments; calibration standards; meter log book; study log book; 1-gal pre-labeled carboys; pre-labeled (20-, 60-, 500- 1000-ml) bottles; filtration equipment; filters; gloves; deionized water; forceps.

Surface water grab at each site:

1. Work downstream to upstream. Measurements will be taken in each riffle or three different locations at the site.
2. Using the water quality instrument(s), take a sub-surface reading of temperature, pH, conductivity, dissolved oxygen, and turbidity.
3. After in-situ readings are recorded, take 1-gal sub-surface grab sample.
4. Place 1-gal sample on ice or refrigerate (approximately 4 °C) until processing.

Processing of Sub-surface grab sample:

Equipment needed: Vacuum pump; 0.45-µm polycarbonate filters; glass fiber filers; pre-labeled bottles; pre-labelled foil packs; RO water; sulfuric acid; graduated cylinders; data sheets.

1. Samples will be filtered at hotel (or on-site trailer) for ammonia (60-ml bottle; 0.45-µm polycarbonate filter). Pre-, and post-filtration blanks should be taken.
2. Samples will be filtered at hotel (or on-site trailer) for chlorophyll *a* and POC using glass fiber filters. Filters will be wrapped in aluminum foil, placed in Ziplock bags, and frozen until analyses. Pre-, and post-filtration blanks should be taken.

3. An aliquot of the sample will be taken at hotel (or on-site trailer) for TN/TP analyses. A 60-ml sample should be placed in 60-ml pre-label containers and frozen until analyses.
4. An aliquot of the sample will be filtered at CERC TSS using nominal pre-weighed glass fiber filters. Filters should be dried immediately after filtration. The aliquot may be placed in a 1000-ml pre-cleaned bottle and preserved with ice or refrigeration prior to filtration. Pre-, and post-filtration blanks should be taken.
5. The remaining water in the sub-surface grab sample will be used for the alkalinity and hardness analyses. The container should be placed on ice or refrigerated until the analyses are conducted (which will be done within 96 hr).
6. Measure the **amount filtered** for ammonia, POC, TSS. Record on data sheet. Acidify ammonia samples with 2 drops of sulfuric acid, if analyses to be run after 24-hr.
7. Chain of Custody (COC) forms for each analyses (e.g., ammonia, TN/TP, TSS) can be filled out daily.

Appendix 6: Field Sampling Filtration Procedure for Surface Water samples for Trace Metals, Cations, Anions, and Dissolved Organic Carbon

A 1-L pre-cleaned LDPE bottle will be used to collect three grab samples per site for trace metals, cations, anions, and dissolved organic carbon. Grab samples should be taken starting downstream and moving upstream. Bottles should be rinsed once with site water prior to the sampling being taken. Bottle should be placed completely under the water surface and filled. Cap bottle underwater, to insure the bottle is as full as possible. Place bottle in cooler with ice, if the filtration procedures cannot be done immediately.

From the 1-L sample, collect one 20-mL filtered sample for the trace metals and cations; a 15-mL sample for anions, and a 20-mL sample for dissolved organic carbon (in that order). Collect each sub-sample using the syringe, FEP sipper straw, and filter cartridge. Filter cartridges for metals and cations, or anions will include a 0.45-µm polyethersulfone (PES) filter and a polypropylene prefilter. Filter cartridges for DOC will include a 0.45-µm polyethersulfone (PES) filter and a glass fiber prefilter. CERC's Chemistry Branch will provide syringe & straw, filter cartridges, 15-mL polypropylene vials for anions, 30-mL bottles for metals/cations, and 40-mL amber glass vials for DOC (all pre-cleaned) for each site, plus a few extras of each for field duplicates).

The syringe/straw and cartridges will be packaged in a single zip-lock. Be sure to place the straw only on a clean surface (e.g., in the zip-lock bag) between subsequent uses for each sub-sample. Wear powderless gloves and throughout the procedure, avoid handling the tip sections of the straws, filter discs, or syringes. After each new sample, the syringe and filter disc are discarded, but the straws are saved for cleaning and reuse. The procedure below is for collection of a 20-mL sample from a larger grab volume.

1. Attach a pre-cleaned sampling straw to the syringe and carefully insert into the grab water sample. Draw the syringe plunger to about 2 ml past the 20-mL mark. Invert syringe and draw plunger to the "stop" to remove all liquid from the straw.
2. Remove the straw and place in a plastic bag for return to the laboratory. Attach a cleaned filter disc (be sure to use the proper cartridge type designated for either A: metals/cations/anionsor B: DOC) and push the plunger first only to the 20-ml mark to expel a few mL of the filtered sample water to waste in order to rinse the filter cartridge with sample.
3. Displace the remaining 20 ml through the filter disc into the appropriate sample container.
4. Discard the syringe and filter cartridge.
5. Cap bottle tightly and if possible, store on ice.
6. Preservative (nitric acid for metals & cations; sulfuric acid for DOC) will be added to each sample upon return to the laboratory, preferably within 96 h after sampling. Samples designated for anions will be kept refrigerated and in the dark (no preservative).

Appendix 7: Field Procedures for In-situ Peeper Sampling of Sediment Pore Water

Diffusion samplers (peepers) are buried 4-6 cm below the sediment surface for a period of 1 to 2 weeks (previous field tests of peepers indicated that equilibration was complete after burial in fine sediments for 4-5 days). The peepers are of a custom CERC design prepared from a 24mL polypropylene snap-cap vial fitted with a 0.45-µm polyethersulfone filter membrane under the cap which has a large hole punched into it to allow water entry. A plastic wire-tie is secured to the body of the vial so the tag end can remain above the sediment surface for retrieval purposes.

Upon retrieval, the peeper vials are rinsed thoroughly with site water and the membrane/perforated cap assembly is carefully removed and replaced with a pre-labeled non-perforated cap. During this process, it is important to avoid contamination of the liquid inside by fine sediment particles on the exterior of the peeper. If visible sediment particles are not readily removed by rinsing with site water, use DI water to rinse the exterior cap region before opening. All samples are placed in racks on ice in the field, and upon return to the laboratory where they are acidified to 1% (v/v) HNO_3.

Appendix 8: List of SOP titles

B4.01 Instrument design, maintenance, and calibration-general

B4.14 Maintenance and Storage of Ross Combination pH Electrode

B4.16 Alkalinity: Burette Method

B4.36 Standard Operating Procedure for the Coulometrics Carbon Model 5020 Analyzer

B4.42 Turbidity Sampling in Water using the Hach Model 2100A Turbidimeter

B4.56 Combination pH Electrode, Ross Sure-flow Model 81-72 Preparation, Maintenance and Storage

B4.62 Orion Model EA940 Expandable Ionanalyzer

B5.03 Preparation of Standard Solutions

B5.13.052693 Procedure for Keeping Fish Culture Records

B5.16 Glassware Washing Procedure for Analytical Biology Section

B5.37 (Revised 2012) Calibration and Operation of the Turner Designs Model 10-AU-006 Fluorometer for the Analyses of Cholorphyll *a* and Pheophytin *a* from Water by In Vitro and In Vivo Methods

B5.40 Invertebrate Glassware Cleaning Procedure

B5.63 Storage, Handling, and Retrieval of Hand-written Material

B5.72.091997 Anesthetization of Fishes for Sampling Purposes

B5.95 Hardness

P.691 Euthanasia and disposal of aquatic organisms (formerly B5.148.030789 Humane Disposal of Fish)

P.683 Humane procedures of anesthetization and handling of research organisms for sampling purposes (formerly B5.154.091997 Humane Procedures for Anesthetization and Handling of Fish or Sampling Purposes)

P.690 Reporting deficiencies in animal care and treatment (formerly B5.160.013189 Reporting Deficiencies in Animal Car and Treatment)

B5.165.091997 Acclimation of Fish to Research Waters

B5.179 Hydrometer Procedure for Particle Size Analysis of Sediment

B5.239 Determining pH of Aqueous Samples with the Orion 290A pH Meter

B5.240.060392 Assignment of Lot Numbers to Fish and Invertebrates

F5.15.020488 Subduing Fish with Ice

F6.2.22 Ammonia Determination using the Orion 290A pH/ISE Meter

F6.2.15 Total Suspended, Fixed Suspended, and Volatile Solid Determination

F6.20.1.082396 Particle Size Determination

P.184 Type and Frequency of Quality Control Measurements Conducted For Elemental Analyses

P.200 Sample Transmittal, Receipt, and Inventory

P.213 Homogenization of Biological Tissue and Sediments By Brittle Fracture

P.221 Mechanical Grinding Of Dried or Semi Dried Tissue Samples with The Bamix Mixer/Blender

P.238 Homogenization of Samples by Manual Procedures

P.239 Data Rejection Criteria and Corrective Action Procedures for Elemental Analyses

P.241 Inductively Coupled Plasma-Mass Spectrometry for Environmental Sample Analysis

P.254 Sample Storage and Disposal Protocol

P.259 Lyophilization and Percent Moisture in Solid Samples

P.510 Multiwave Microwave Acid Digestion of Environmental Samples

P.566 Syringe and Disc Filtration of Water or Pore Water Samples for Trace Element Determinations

P.570 Operation, Calibration, and Data Transfer of the YSI650 Multi-parameter Display System in Conjunction with the YSI6600 Multi-parameter Water Quality Monitor Unit

P.578 Collection of Benthic Macroinvertebrate Samples For Measurement of Trace Elements

P.579 Sampling Sediment for Elemental Analysis

P.636 MULTIWAVE 3000 Microwave Acid Digestion of Environmental Samples

P.705 Determination of Fluoride, Chloride, Nitrite, Nitrate, Bromide, and Sulfate in Water by Ion Chromatography with a Dionex ICS-1100

P.722 Determination of Total Organic Carbon in Water with the Shimadzu TOC-L Analyzer

Appendix 9: CERC LIVE ORGANISM TRANSFER REQUEST (LOTR)

Revision Date: 9/4/14

DATE: **ESTIMATED DATE OF ORGANISM TRANSFER:**

COMPLETED BY: **CERC CONTACT:**

ORGANISMS TO BE TRANSFERRED:

REASON FOR ORGANISMS TO BE TRANSFERRED:

CERC STUDY PLAN # OR OTHER:

AGENCY OR COMPANY ASSISTING TRANSFER:

SIZE/AGE OF ORGANISMS:

QUANTITY:

WILD OR CAPTIVE:

ORIGIN:

DESTINATION (if not in the quarantine room, justify the reason):

CERC BIOSECURITY DESIGNATION (IF DETERMINED) AND JUSTIFICATION FOR EACH OF THE FOLLOWING CATEGORIES:

In the context of potential for release of non-native target organisms into the Missouri River watershed:

In the context of potential for release of non-native, non-target organisms to the CERC or into the Missouri River watershed:

In the context of potential for transfer or release of harmful pathogens to the CERC or into the Missouri River watershed:

KNOWN BIOSECURITY CONCERNS:

BIOSECURITY PLAN:

APPROVED BY: **DATE:**

Appendix 10: CERC FISH TRANSFER STANDARD TREATMENT PROCEDURE

The following treatment procedure is recommended for all incoming fish transfers at CERC. This treatment should be used as a minimum procedure; additional processes may be applicable or required depending on the source of the fish and potential biosecurity risks associated with them. This treatment procedure has several purposes. First, it should allow the "hauling" water to be rinsed away from the fish and reduce the transfer of potential pathogens in the haul water. Second, the high concentration of salt will aid in killing or reducing external parasites from the fish's body. Finally, it will allow potential non-target organisms to separate away from the fish and allow a visual period to observe the batch of fish for non-target organisms.

The treatment concentration may need to be lowered based on species and age of fish. It is recommended to test the treatment first with a single specimen and observe for signs of stress. Lower salt concentrations or shorter submersion times can still be effective at accomplishing the purpose of this treatment.

1) Separate dip nets should be used to transfer fish between hauling tank and treatment tank as well as between treatment tanks or nets should be placed in the salt bath with the fish.

2) Net crayfish out of the hauling tank and put into a 25 ppt [fish treatment =0 ppt (38g/gal or 10 g/L)] sodium chloride (NaCl) solution bath (well water) for 1 minute, then transfer crayfish to 50 ppt NaCl solution for four minutes. Following the first cycle of salt treatment, crayfish are placed into well water for five minutes. During this time, monitor the holding container for non-target organisms that may be at bottom of the tank or separated from the target organisms. Visible non-target organisms should be removed from the tank and euthanized immediately.

3) After the first cycle is completed, transfer the crayfish to another 25 ppt NaCl solution bath for an additional one minute; into 50 ppt NaCl for four minutes; into well water for five minutes. Again during this time, monitor the holding container for non-target organisms that may be at the bottom of the tank or separated from the target organisms. Non-target organisms should be removed from the tank and euthanized immediately.

4) After the second salt dip treatment, crayfish can be transferred to the appropriate rearing place at CERC.

5) At the completion of the salt water dip, the treatment tank solutions will be treated with a 200 mg/L chlorine solution for a minimum of one hour. The solution can then be neutralized with sodium thiosulfate (5.3g/gal) before being discharged if appropriate.

6) All water contained in the hauling tank or containers will be treated with 200 mg/L chlorine solution for a minimum of one hour before it is dumped into the sand trap behind the east boat barn.

7) The hauling tank or containers will then be surface sprayed with a disinfectant (200 mg/L bleach spray or 2% Virkon Aquatic®, recommended), rinsed and dried thoroughly for future use.

8) All equipment (waders, nets, buckets) associated with the fish transfer should be sprayed with an appropriate disinfectant (200 mg/L bleach spray or 2% Virkon Aquatic®, recommended), at the completion of the transfer.

9) All personnel involved in the transferring process will be instructed to follow this procedure.

Note: Salt treatment of crayfish was modified for second batch of ovigerous females (Johnson et al. 2003).

1) Separate dip nets should be used to transfer fish between hauling tank and treatment tank as well as between treatment tanks or nets should be placed in the salt bath with the fish.

2) Net crayfish out of the hauling tank and put into a 10 ppt sodium chloride (NaCl) solution bath (well water) for 10 minutes, then transfer crayfish to well water for five minutes. During this time, monitor the holding container for non-target organisms that may be at bottom of the tank or separated from the target organisms. Visible non-target organisms should be removed from the tank and euthanized immediately.

3) After the first cycle is completed, transfer the crayfish to another 10 ppt NaCl solution bath for an additional 10 minutes, then into well water for five minutes. Again during this time, monitor the holding container for non-target organisms that may be at the bottom of the tank or separated from the target organisms. Non-target organisms should be removed from the tank and euthanized immediately.

Appendix 11: CERC Hazard Analysis and Critical Control Point Plans (HACCPS)

CERC Biological Sample HACCP Final Plan

HACCP Step 1 – Activity Description

Management Objective & Contact Information	
HACCP Plan Title: Importation of field-collected fresh or frozen, non-living biologic samples for toxic organic and inorganic chemical analysis	
Management Objective: Import field-collected fresh or frozen non-living biologic samples to the Columbia Environmental Research Center (CERC) for analysis of toxic organic and inorganic compounds. Provide this information to resource managers and regulatory agencies concerned about negative impacts on ecosystem health.	Contact Person: David Alvarez Phone: 573-441-2970 Email: dalvarez@usgs.gov

Activity Description

i.e. Who; What; Where; When; How; Why

Who: Environmental Chemistry Branch
What: Receiving fresh or frozen, non-living biologic samples for analysis of organic and inorganic compounds
Where: CERC loading dock and quarantine room
When: Throughout the year
How: Biologic samples are received from collaborative partners via shipping carriers (Fedex , UPS, etc.) or collected by CERC personnel and transported to the lab and unloaded on the back loading dock. Cooler is taken to the quarantine room, opened up; an inventory of the samples is taken and if needed, sample containers and coolers are cleaned at this time. Samples are then typically stored in a walk-in freezer for later analysis.
Why: To provide scientific data on the presence and/or concentrations of chemical constituents to collaborative partners such as state and federal agencies.

HACCP Step 2 – Activity Flow Chart

Outline Sequential Tasks of Activity

Task 1	Title: Coordination of sampling and shipping logistics.
	Description: Designated person coordinates with the partner agency to ensure that samples are collected and stored properly according to CERC Standard Operating Procedures (SOPs) and Chain of Custody (COC) forms if necessary. Informs staff members of approximate arrival time, intended purpose of the samples and designates appropriate staff to handle samples. Information related to potential biological non-targets at the sampling site and sample receiving are discussed.

Task 2	Title: Shipping the samples and receiving of the shipment at CERC.
	Description: Samples are packaged in a cooler by field crews and shipped by a shipping carrier. Upon arrival at CERC, Samples are off-loaded by shipping carrier onto the back loading dock or occasionally at the front desk. Front desk notifies the recipient that the shipment has arrived.

Task 3	Title: Processing and handling the shipment.
	Description: Cooler is opened up and an inventory of the samples is taken. If sample jars have broken, as much sample that can be recovered is transferred into a new container. Any residual water or sediment on the inside or outside of the cooler is removed. Both the sample jars and the cooler is cleaned thoroughly inside and out.

Task 4	Title: Repacking and storage of the samples
	Description: Samples are either repacked in the cooler or placed in separate storage containers (boxes, tubs, freezer cages, etc.) and taken to the proper location for storage or direct processing of the samples.

Task 5	Title: Processing and analyzing the samples.
	Description: Samples are processed in the lab according to the appropriate standard operating procedure. Excess sample will be disposed of according to CERC's hazardous substances and biosecurity guidelines.

HACCP Step 3 – Identify Potential Non-Targets

Non-Targets That May Potentially Be Moved/Introduced
Vertebrates: None

Invertebrates: None
Plants: None
Other Organisms (pathogens, parasites, etc.) Bacteria, parasites and pathogens of concern to both human health and aquatic organisms; specifically *Escherichia coli* (*E. coli*), hepatitis, Aeromonas/Pseudomonas, *Batrachochytrium dendrobatidis* (chytrid fungus)

1 **Tasks** (From Step 2)	2 **Potential Non-Targets** (From Step 3)	3 **Risk Assessment** Are any non-targets significant? Yes or No	4 **Justification** Justify your answer in Column 3	5 **Control** What Control Measures Can be applied during this task to reduce the risk of non-targets?	6 **CCP** Is this task a CCP? Yes or No	7 **Justification** Justify your answer in column 6
Task #1 Title: Coordination of sampling and shipping logistics.	**Vertebrates:** None	No	There is no risk of transferring non-target species because samples have not been shipped yet.	N/A	No	There are no significant non-targets during this task
	Invertebrates: None	No				
	Plants: None	No				
	Other Organisms Bacteria and pathogens of concern to both human health and aquatic organisms.	No				

1 **Tasks** (From Step 2)	2 **Potential Non-Targets** (From Step 3)	3 **Risk Assessment** Are any non-targets significant? Yes or No	4 **Justification** Justify your answer in Column 3	5 **Control** What Control Measures Can be applied during this task to reduce the risk of non-targets?	6 **CCP** Is this task a CCP? Yes or No	7 **Justification** Justify your answer in column 6
Task #2 Title: Receiving of the shipment at CERC.	**Vertebrates:** None	No	There is a very low risk of transferring non-target species because samples are contained in coolers and kept sealed.	N/A	No	There are no significant non-targets during this task
	Invertebrates: None	No				
	Plants: None	No				
	Other Organisms Bacteria and pathogens of concern to both human health and aquatic organisms.	No				

1 **Tasks** (From Step 2)	2 **Potential Non-Targets** (From Step 3)	3 **Risk Assessment** Are any non-targets significant? Yes or No	4 **Justification** Justify your answer in Column 3	5 **Control** What Control Measures Can be applied during this task to reduce the risk of non-targets?	6 **CCP** Is this task a CCP? Yes or No	7 **Justification** Justify your answer in column 6
Task #3 Title: Processing and handling the shipment.	**Vertebrates:** None	No	Once samples are on-station at CERC, the chance for non-target species to be transferred to an environment where they can spread or thrive is highly likely.	If samples are not processed immediately, the cooler and its entire contents should be placed in the walk-in freezer. Once removed from the freezer, the following procedures must be followed. Start by preparing the sample processing table in the quarantine room. Clean off sufficient space and place bench paper on the work area. Carry the cooler directly from the loading dock into the quarantine room. Gloves should be worn while handling the shipment. Sample bottles should be removed from the cooler, dried off and sprayed down with a 2% Virkon® solution and allowed to sit for ten minutes before it is rinsed and wiped dry. Excess water in the cooler should be poured into a bucket dumping station. Excess sediment should be removed with gloved hands and paper towels, placed in a sealed ziplock bag and placed in the trash can. The cooler should then be sprayed with 2% Virkon® and allowed to sit for ten minutes before it is rinsed and wiped dry. The samples can either be repacked in the same cooler or transferred to a clean container and then removed from the room. Bench paper should be rolled up and placed into the trash can. All work surfaces should be cleaned with Virkon® spray and paper towels.	Yes	This is a critical control point because this is the point where the transfer of a non-target species is most likely to occur. Surfaces that tissue samples are processed on has the highest risk of introducing a non-target species to CERC.
	Invertebrates: None	No				
	Plants: None	No				
	Other Organisms Bacteria and pathogens of concern to both human health and aquatic organisms.	Yes				

1 **Tasks** (From Step 2)	2 **Potential Non-Targets** (From Step 3)	3 **Risk Assessment** Are any non-targets significant? Yes or No	4 **Justification** Justify your answer in Column 3	5 **Control** What Control Measures can be applied during this task to reduce the risk of non-targets?	6 **CCP** Is this task a CCP? Yes or No	7 **Justification** Justify your answer in column 6
Task #4 Title: Storage of the samples	**Vertebrates:** None	No	Risk is low for transferring non-target species because samples and storage cooler have been cleaned thoroughly before storage. Storage of samples in sealed containers in either a cooler or freezer minimizes any potential transfer of pathogens.	N/A	No	There are no significant non-targets during this task.
	Invertebrates: None	No		N/A		
	Plants: None	No		N/A		
	Other Organisms Bacteria and pathogens of concern to both human health and aquatic organisms.	No		N/A		

1 **Tasks** (From Step 2)	2 **Potential Non-Targets** (From Step 3)	3 **Risk Assessment** Are any non-targets significant? Yes or No	4 **Justification** Justify your answer in Column 3	5 **Control** What Control Measures Can be applied during this task to reduce the risk of non-targets?	6 **CCP** Is this task a CCP? Yes or No	7 **Justification** Justify your answer in column 6
Task #5 Title: Processing and analyzing the samples.	**Vertebrates:** None	No	If work area is not thoroughly disinfected after processing the samples, the risk of transferring pathogens throughout the lab is present.	During the processing of samples, Best Lab Practices (BLP's) should be used at all times including wearing gloves while handling the samples. Upon completion of sample processing, all work and floor surfaces and equipment used during the processing must be sprayed down with a 2% Virkon® Aquatic or 2% chlorine solution and allowed to sit for ten minutes before surfaces are rinsed. Excess tissue samples can be placed in a bag and disposed of in the trash following a period of time after the final publication of data as agreed upon by CERC and its collaborator. For samples that cannot be disposed of (e.g., NRDAR projects), arrangements should be made for long-term storage at CERC or another facility during the planning stages of the project.	Yes	Samples will be exposed and handled on work surfaces potentially allowing pathogens to be transferred throughout the center and potentially infecting existing cultures or research projects.
	Invertebrates: None	No				
	Plants: None	No				
	Other Organisms Bacteria and pathogens of concern to both human health and aquatic organisms.	Yes				

HACCP Step 5 – Non-Target Risk Action Plan (NTRAP)

(Use this form for any "Yes" from Column 6 of HACCP Step 4 - Non-Target Analysis Worksheet) One page for each Critical Control Point				
Mangement Objective From Step 1	Import field-collected non-living biologic samples to the Columbia Environmental Research Center (CERC) for analysis of toxic organic and inorganic compounds that may negatively influence ecosystems. Provide this information to resource managers and regulatory agencies.			
Critical Control Point:	**Task #**	3	**Title:**	Processing and handling the shipment.
Significant Non-Target(s) (Step 4, Column 3)	Bacteria, parasites and pathogens of concern to both human health and aquatic organisms; specifically *Escherichia coli* (*E. coli*), hepatitis, Aeromonas/Pseudomonas, *Batrachochytrium dendrobatidis* (chytrid fungus)			
Control Measure(s) (Step 4, Column 5)	Start by preparing the sample processing table in the quarantine room. Clean off sufficient space and place bench paper on the work area. Carry the cooler directly from the loading dock into the quarantine room. Gloves should be worn while handling the shipment. Sample bottles should be removed from the cooler, dried off and sprayed down with 2% Virkon® and allowed to sit for ten minutes before it is rinsed and wiped dry. Excess water in the cooler should be poured into a bucket dumping station. Excess sediment should be removed with gloved hands and paper towels, put in a sealed ziplock bag and placed in the trash can. The cooler should then be sprayed with 2% Virkon® and allowed to sit for ten minutes before it is rinsed and wiped dry. The samples can either be repacked in the same cooler or transferred to a clean container and then removed from the room. Bench paper should be rolled up and placed into the trash can. All work surfaces should be cleaned with Virkon® spray and paper towels.			
Precribed ranges, limits, or citeria for control measure(s): (PRLC)	Sample bottles and cooler should be sprayed or wiped with 2% Virkon® for 10 minutes before rinsing or drying. Care must be taken to not damage sample labels. Coolers should be visually inspected after cleaning so there is no visible mud or sediment remaining outside or inside the cooler.			
Monitoring the Control Measure(s)	**Who?**	Chemistry technicians		
	How?	Make sure all surfaces have been cleaned and sprayed with 2% Virkon and allowed to sit for ten minutes.		
	Where?	Quarantine room		
	How often?	When receiving a shipment of tissue samples.		
Corrective Action(s) if Control	Repeat control measures from task 3. Remove all visible dirt, sediments and water from cooler and spray with 2% Virkon®.			

Measures Fail (or PRLC cannot be met)	
Supporting Documents *(For example, Management Plan, Checklist, Decontamination Techniques, SOPs, Scientific Journal Articles, etc.)*	

HACCP Step 5 – Non-Target Risk Action Plan (NTRAP)

(Use this form for any "Yes" from Column 6 of HACCP Step 4 - Non-Target Analysis Worksheet)
One page for each Critical Control Point

Mangement Objective From Step 1	Import field-collected non-living biologic samples to the Columbia Environmental Research Center (CERC) for analysis of toxic organic and inorganic compounds that may negatively influence ecosystems. Provide this information to resource managers and regulatory agencies.			
Critical Control Point:	**Task #**	5	**Title:**	Processing and analyzing the samples.
Significant Non-Target(s) (Step 4, Column 3)	Bacteria and pathogens of concern to both human health and aquatic organisms.			
Control Measure(s) (Step 4, Column 5)	During the processing of samples, Best Lab Practices (BLP's) should be used at all times including wearing gloves while handling the samples. Upon completion of sample processing, all work and floor surfaces and equipment used during the processing must be sprayed down with a 2% Virkon® Aquatic solution or a 2% chlorine solution and allowed to sit for ten minutes before surfaces are rinsed.			
Precribed ranges, limits, or citeria for control measure(s): (PRLC)	All work surfaces and equipment should be sprayed or wiped with 2% Virkon® or 2% chlorine solution for 10 minutes before rinsing or drying.			
Monitoring the Control Measure(s)	**Who?**	Chemistry technicians		
	How?	Make sure all surfaces have been cleaned and sprayed with 2% Virkon or 2% chlorine and allowed to sit for ten minutes.		
	Where?	Grinding room		

	How often?	When receiving a shipment of tissue samples.
Corrective Action(s) if Control Measures Fail (or PRLC cannot be met)	Repeat control measures from task 5. Remove all visible dirt, sediments and water from cooler and spray with 2% Virkon® or 2% chlorine.	
Supporting Documents *(For example, Management Plan, Checklist, Decontamination Techniques, SOPs, Scientific Journal Articles, etc.)*		

Development Team Members		**James Candrl and David Alvarez**	
Date Developed:	**11/20/14**	**Date(s) Reviewed:**	**12/29/14 Biosecurity Committee**

***all fields in grey are required**

CERC Water Sample HACCP final Plan

HACCP Step 1 – Activity Description

Management Objective & Contact Information	
HACCP Plan Title: Importation of water samples for organic and inorganic chemistry analysis	
Management Objective: Import field-collected water samples to the Columbia Environmental Research Center (CERC) for analysis of organic and inorganic compounds. Provide this information to resource managers and regulatory agencies concerned about negative impacts on ecosystem health.	Contact Person: David Alvarez Phone: 573-441-2970 Email: dalvarez@usgs.gov

Activity Description

i.e. Who; What; Where; When; How; Why

Who: Environmental Chemistry Branch
What: Receiving water samples for analysis of organic and inorganic compounds
Where: CERC loading dock and quarantine room
When: Throughout the year
How: Water samples are received from collaborative partners via shipping carriers (Fedex , UPS, etc.) or collected by CERC personnel and transported to the lab and unloaded on the back loading dock. Cooler is taken to the quarantine room, opened up; an inventory of the samples is taken and if needed, sample containers and coolers are cleaned at this time. Samples are then typically stored in a walk-in freezer for later analysis.
Why: To provide scientific data on the presence and/or concentrations of chemical constituents to collaborative partners such as state and federal agencies.

HACCP Step 2 – Activity Flow Chart

Outline Sequential Tasks of Activity

Task 1	Title: Coordination of sampling and shipping logistics.
	Description: Designated person coordinates with the partner agency to ensure that samples are collected and stored properly according to CERC Standard Operating Procedures (SOPs) and Chain of Custody (COC) forms if necessary. Informs staff members of approximate arrival time, intended purpose of the samples and designates appropriate staff to handle samples. Information related to potential biological non-targets at the sampling site and sample receiving are discussed.

Task 2	Title: Shipping the samples and receiving of the shipment at CERC.
	Description: Samples are packaged in a cooler by field crews and shipped by a shipping carrier. Upon arrival at CERC, Samples are off-loaded by shipping carrier onto the back loading dock or occasionally at the front desk. Front desk notifies the recipient that the shipment has arrived.

Task 3	Title: Processing and handling the shipment.
	Description: Cooler is opened up and an inventory of the samples is taken. If sample jars have broken, as much sample that can be recovered is transferred into a new container. Any residual water or sediment on the inside or outside of the cooler is removed, and discarded/treated properly. Both the sample jars and the cooler is cleaned thoroughly inside and out.

Task 4	Title: Repacking and storage of the samples
	Description: Samples are either repacked in the cooler or placed in separate storage containers (boxes, tubs, freezer cages, etc.) and taken to the proper location for storage or direct processing of the samples.

Task 5	Title: Processing and analyzing the samples.
	Description: Samples are processed in the lab according to the appropriate standard operating procedure. Excess sample will be disposed of according to CERC's hazardous substances and biosecurity guidelines.

HACCP Step 3 – Identify Potential Non-Targets

Non-Targets That May Potentially Be Moved/Introduced
Vertebrates: Small fish of various species
Invertebrates: Numerous invertebrates (worms, crayfish, leeches, barnacles) could be potentially be transferred but specifically New Zealand Mud Snails (*Potamopyrgus antipodarum*), zebra mussel

(*Dreissena polymorpha*) and quagga mussel (*Dreissena bugensis*)veligers.
Plants: Water meal and algae
Other Organisms (pathogens, parasites, etc.) Bacteria, parasites and pathogens of concern to both human health and aquatic organisms; specifically *Escherichia coli* (*E. coli*) and hepatitis.

1 **Tasks** (From Step 2)	2 **Potential Non-Targets** (From Step 3)	3 **Risk Assessment** Are any non-targets significant? Yes or No	4 **Justification** Justify your answer in Column 3	5 **Control** What Control Measures Can be applied during this task to reduce the risk of non-targets?	6 **CCP** Is this task a CCP? Yes or No	7 **Justification** Justify your answer in column 6
Task #1 Title: Coordination of sampling and shipping logistics.	**Vertebrates:** Small fish of various species	No	There is no risk of transferring non-target species because samples have not been shipped yet.	N/A	No	There are no significant non-targets during this task
	Invertebrates: Numerous invertebrates (worms, crayfish, leeches, barnacles) but specifically New Zealand Mud Snails (*Potamopyrgus antipodarum*), zebra mussel (*Dreissena polymorpha*)and quagga mussel (*Dreissena bugensis*)veligers.	No				
	Plants: water meal and algae	No				
	Other Organisms Bacteria, parasites and pathogens of concern to both human health and aquatic organisms; specifically *Escherichia coli* (*E. coli*) and hepatitis.	No				

1 **Tasks** (From Step 2)	2 **Potential Non-Targets** (From Step 3)	3 **Risk Assessment** Are any non-targets significant? Yes or No	4 **Justification** Justify your answer in Column 3	5 **Control** What Control Measures Can be applied during this task to reduce the risk of non-targets?	6 **CCP** Is this task a CCP? Yes or No	7 **Justification** Justify your answer in column 6
Task #2 Title: Receiving of the shipment at CERC.	**Vertebrates:** Small fish of various species	No	Shipping trucks, warehouses and CERC loading dock are dry, so environment for aquatic species to survive and spread is not likely.	N/A	No	There are no significant non-targets during this task
	Invertebrates: Numerous invertebrates (worms, crayfish, leeches, barnacles) but specifically New Zealand Mud Snails (*Potamopyrgus antipodarum*), zebra mussel (*Dreissena polymorpha*)and quagga mussel (*Dreissena bugensis*)veligers.	No				
	Plants: water meal and algae	No				
	Other Organisms Bacteria, parasites and pathogens of concern to both human health and aquatic organisms; specifically *Escherichia coli* (*E. coli*) and hepatitis.	No				

1 **Tasks** (From Step 2)	2 **Potential Non-Targets** (From Step 3)	3 **Risk Assessment** Are any non-targets significant? Yes or No	4 **Justification** Justify your answer in Column 3	5 **Control** What Control Measures Can be applied during this task to reduce the risk of non-targets?	6 **CCP** Is this task a CCP? Yes or No	7 **Justification** Justify your answer in column 6
Task #3 Title: Processing and handling the shipment.	**Vertebrates:** Small fish of various species	Yes	Once samples are on-station at CERC, the chance for non-target species to be transferred to an environment where they can spread or thrive is highly likely.	If samples are not processed immediately, the cooler and its entire contents should be placed in the walk-in freezer. Once removed from the freezer, the following procedures must be followed. If freezing is not advised due to risk sample containers breaking (a potential risk with very wet sediments in glass jars), then the outside of the cooler should be decontaminated in the quarantine room using a 2% Virkon® solution and then the cooler and its entire contents can be placed in the walk-in cooler. Start by preparing the sample processing table in the quarantine room. Clean off sufficient space and place bench paper on the work area. Carry the cooler directly from the loading dock into the quarantine room. Gloves should be worn while handling the shipment. Sample bottles should be removed from the cooler, dried off and sprayed down with a 2% Virkon® solution and allowed to sit for ten minutes before it is rinsed and wiped dry. Excess water in the cooler should be poured into a bucket dumping station. Excess sediment should be removed with gloved hands and paper towels, placed in a sealed ziplock bag and placed in the trash can. The cooler should then be sprayed with 2% Virkon® and allowed to sit for ten minutes before it is rinsed and wiped dry. The samples can either be repacked in the same cooler or transferred to a clean container and then removed from the room. Bench paper should be rolled up and placed into the trash can. All work surfaces should be cleaned with Virkon® spray and paper towels.	Yes	This is a critical control point because this is the point where the transfer of a non-target species is most likely to occur. Loose sediments or excess water in the cooler has the highest risk of introducing a non-target species to CERC.
	Invertebrates: Numerous invertebrates (worms, crayfish, leeches, barnacles) but specifically New Zealand Mud Snails (*Potamopyrgus antipodarum*), zebra mussel (*Dreissena polymorpha*)and quagga mussel (*Dreissena bugensis*)veligers.	Yes				
	Plants: water meal and algae	Yes				
	Other Organisms Bacteria, parasites and pathogens of concern to both human health and aquatic organisms; specifically *Escherichia coli* (*E. coli*) and hepatitis.	Yes				

1 **Tasks** (From Step 2)	2 **Potential Non-Targets** (From Step 3)	3 **Risk Assessment** Are any non-targets significant? Yes or No	4 **Justification** Justify your answer in Column 3	5 **Control** What Control Measures can be applied during this task to reduce the risk of non-targets?	6 **CCP** Is this task a CCP? Yes or No	7 **Justification** Justify your answer in column 6
Task #4 Title: Storage of the samples	**Vertebrates:** Small fish of various species	No	Risk is low for transferring non-target species because samples and storage cooler have been cleaned thoroughly before storage. Storage of samples in closed bottles in either a cooler or freezer does not allow for non-target species to remain viable.	N/A	No	There are no significant non-targets during this task.
	Invertebrates: Numerous invertebrates (worms, crayfish, leeches, barnacles) but specifically New Zealand Mud Snails (*Potamopyrgus antipodarum*), zebra mussel (*Dreissena polymorpha*)and quagga mussel (*Dreissena bugensis*)veligers.	No		N/A		
	Plants: water meal and algae	No		N/A		
	Other Organisms Bacteria, parasites and pathogens of concern to both human health and aquatic organisms; specifically *Escherichia coli* (*E. coli*) and hepatitis.	No		N/A		

1 **Tasks** (From Step 2)	2 **Potential Non-Targets** (From Step 3)	3 **Risk Assessment** Are any non-targets significant? Yes or No	4 **Justification** Justify your answer in Column 3	5 **Control** What Control Measures Can be applied during this task to reduce the risk of non-targets?	6 **CCP** Is this task a CCP? Yes or No	7 **Justification** Justify your answer in column 6

Task #5 Title: Processing and analyzing the samples.	**Vertebrates:** Small fish of various species	No	The risk is low for transferring non-target species to CERC during this task. If samples have not previously been frozen or stored in an anaerobic environment for a period of time, they are processed using strict controls to minimize the risk for release of any non-target organisms	Excess water samples can be stored for a period suitable for the analytes of interest. Following this time, any un-used water should be disposed of by pouring down the quarantine room waste system for treatment.	No	There are no significant non-targets during this task.
	Invertebrates: Numerous invertebrates (worms, crayfish, leeches, barnacles) but specifically New Zealand Mud Snails (*Potamopyrgus antipodarum*), zebra mussel (*Dreissena polymorpha*)and quagga mussel (*Dreissena bugensis*)veligers.	No				
	Plants: water meal and algae	No				
	Other Organisms Bacteria, parasites and pathogens of concern to both human health and aquatic organisms; specifically *Escherichia coli* (*E. coli*) and hepatitis.	No				

C. HACCP Step 5 – Non-Target Risk Action Plan (NTRAP)

(Use this form for any "Yes" from Column 6 of HACCP Step 4 - Non-Target Analysis Worksheet)
One page for each Critical Control Point

Mangement Objective From Step 1	Import water samples to the Columbia Environmental Research Center (CERC) for analysis of organic and inorganic compounds that may negatively influence ecosystems. Provide this information to management and regulatory agencies.			
Critical Control Point:	**Task #**	3	**Title:**	Processing and handling the shipment.
Significant Non-Target(s) (Step 4, Column 3)	Small fish of various species, numerous small invertebrates (worms, crayfish, leeches) but specifically New Zealand Mud Snails, zebra and quagga mussel veligers, water meal, algae, numerous pathogens of concern to both human health (*E. coli,hepatitis*) and aquatic species.			
Control Measure(s) (Step 4, Column 5)	Start by preparing the sample processing table in the quarantine room. Clean off sufficient space and place bench paper on the work area. Carry the cooler directly from the loading dock into the quarantine room. Gloves should be worn while handling the shipment. Sample bottles should be removed from the cooler, dried off and sprayed down with 2% Virkon® and allowed to sit for ten minutes before it is rinsed and wiped dry. Excess water in the cooler should be poured into a bucket dumping station. Excess sediment should be removed with gloved hands and paper towels, put in a sealed ziplock bag and placed in the trash can. The cooler should then be sprayed with 2% Virkon® and allowed to sit for ten minutes before it is rinsed and wiped dry. The samples can either be repacked in the same cooler or transferred to a clean container and then removed from the room. Bench paper should be rolled up and placed into the trash can. All work surfaces should be cleaned with Virkon® spray and paper towels.			
Precribed ranges, limits, or citeria for control measure(s): (PRLC)	Sample bottles and cooler should be sprayed or wiped with 2% Virkon® for 10 minutes before rinsing or drying. Care must be taken to not damage sample labels. Coolers should be visually inspected after cleaning so there is no visible mud or sediment remaining outside or inside the cooler.			
Monitoring the Control Measure(s)	**Who?**	Chemistry technicians		
	How?	Make sure all surfaces have been cleaned and sprayed with 2% Virkon and allowed to sit for ten minutes.		
	Where?	Quarantine room		
	How often?	When receiving a shipment of water samples.		
Corrective Action(s) if Control Measures Fail	Repeat control measures from task 3. Remove all visible dirt, sediments and water from cooler and spray with 2% Virkon®.			

(or PRLC cannot be met)			
Supporting Documents *(For example, Management Plan, Checklist, Decontamination Techniques, SOPs, Scientific Journal Articles, etc.)*			
Development Team Members	**James Candrl and David Alvarez**		
Date Developed:	**11/7/14**	**Date(s) Reviewed:**	**12/29/14 Biosecurity Committee**

***all fields in grey are required**

CERC Sediment Sample HACCP final Plan

HACCP Step 1 – Activity Description

Management Objective & Contact Information	
HACCP Plan Title: Importation of field-collected soil and sediment samples for organic and inorganic chemistry analysis	
Management Objective: Import field-collected soil and sediment samples to the Columbia Environmental Research Center (CERC) for analysis of organic and inorganic compounds. Provide this information to resource managers and regulatory agencies concerned about negative impacts on ecosystem health.	Contact Person: David Alvarez Phone: 573-441-2970 Email: dalvarez@usgs.gov

Activity Description
i.e. Who; What; Where; When; How; Why

Who: Environmental Chemistry Branch
What: Receiving soil and sediment samples for analysis of organic and inorganic compounds
Where: CERC loading dock and quarantine room
When: Throughout the year
How: Soil and sediment samples are received from collaborative partners via shipping carriers (Fedex , UPS, etc.) or collected by CERC personnel and transported to the lab and unloaded on the back loading dock. Cooler is taken to the quarantine room, opened up; an inventory of the samples is taken and if needed, sample containers and coolers are cleaned at this time. Samples are then typically stored in a walk-in freezer for later analysis.
Why: To provide scientific data on the presence and/or concentrations of chemical constituents to collaborative partners such as state and federal agencies.

HACCP Step 2 – Activity Flow Chart

Outline Sequential Tasks of Activity

Task 1	Title: Coordination of sampling and shipping logistics.
	Description: Designated person coordinates with the partner agency to ensure that samples are collected and stored properly according to CERC Standard Operating Procedures (SOPs) and Chain of Custody (COC) forms if necessary. Informs staff members of approximate arrival time, intended purpose of the samples and designates appropriate staff to handle samples. Information related to potential biological non-targets at the sampling site and sample receiving are discussed.

Task 2	Title: Shipping the samples and receiving of the shipment at CERC.
	Description: Samples are packaged in a cooler by field crews and shipped by a shipping carrier. Upon arrival at CERC, Samples are off-loaded by shipping carrier onto the back loading dock or occasionally at the front desk. Front desk notifies the recipient that the shipment has arrived.

Task 3	Title: Processing and handling the shipment.
	Description: Cooler is opened up and an inventory of the samples is taken. If sample jars have broken, as much sample that can be recovered is transferred into a new container. Any residual water or sediment on the inside or outside of the cooler is removed, and discarded/treated properly. Both the sample jars and the cooler is cleaned thoroughly inside and out.

Task 4	Title: Repacking and storage of the samples
	Description: Samples are either repacked in the cooler or placed in separate storage containers (boxes, tubs, freezer cages, etc.) and taken to the proper location for storage or direct processing of the samples.

Task 5	Title: Processing and analyzing the samples.
	Description: Samples are processed in the lab according to the appropriate standard operating procedure. Excess sample will be disposed of according to CERC's hazardous substances and biosecurity guidelines.

HACCP Step 3 – Identify Potential Non-Targets

Non-Targets That May Potentially Be Moved/Introduced
Vertebrates: Small fish of various species, amphibians, fish and amphibian eggs depending on time of year

Invertebrates: Numerous invertebrates (worms, crayfish, leeches, barnacles) could be potentially be transferred but specifically New Zealand Mud Snails (*Potamopyrgus antipodarum*), zebra mussel (*Dreissena polymorpha*) and quagga mussel (*Dreissena bugensis*)veligers. Various aquatic insects and/or eggs, fire ants (*Solenopsis* sp.)
Plants: Family Lemnaceae **(**Water meal *Wolffia and Wolfiella* spp. and Duckweed, Lemna spp.)
Other Organisms (pathogens, parasites, etc.) Bacteria, parasites and pathogens of concern to both human health and aquatic organisms; specifically *Escherichia coli* (*E. coli*), hepatitis, Aeromonas/Pseudomonas, *Batrachochytrium dendrobatidis* (chytrid fungus)

1 **Tasks** (From Step 2)	2 **Potential Non-Targets** (From Step 3)	3 **Risk Assessment** Are any non-targets significant? Yes or No	4 **Justification** Justify your answer in Column 3	5 **Control** What Control Measures Can be applied during this task to reduce the risk of non-targets?	6 **CCP** Is this task a CCP? Yes or No	7 **Justification** Justify your answer in column 6
Task #1 Title: Coordination of sampling and shipping logistics.	**Vertebrates:** Small fish of various species	No	There is no risk of transferring non-target species because samples have not been shipped yet.	N/A	No	There are no significant non-targets during this task
	Invertebrates: Numerous invertebrates (worms, crayfish, leeches, barnacles) but specifically New Zealand Mud Snails (*Potamopyrgus antipodarum*), zebra mussel (*Dreissena polymorpha*)and quagga mussel (*Dreissena bugensis*)veligers.	No				
	Plants: water meal	No				
	Other Organisms Bacteria, parasites and pathogens of concern to both human health and aquatic organisms; specifically *Escherichia coli* (*E. coli*) and hepatitis.	No				

1 **Tasks** (From Step 2)	2 **Potential Non-Targets** (From Step 3)	3 **Risk Assessment** Are any non-targets significant? Yes or No	4 **Justification** Justify your answer in Column 3	5 **Control** What Control Measures Can be applied during this task to reduce the risk of non-targets?	6 **CCP** Is this task a CCP? Yes or No	7 **Justification** Justify your answer in column 6
Task #2 Title: Receiving of the shipment at CERC.	**Vertebrates:** Small fish of various species	No	Shipping trucks, warehouses and CERC loading dock are dry, so environment for aquatic species to survive and spread is not likely.	N/A	No	There are no significant non-targets during this task
	Invertebrates: Numerous invertebrates (worms, crayfish, leeches, barnacles) but specifically New Zealand Mud Snails (*Potamopyrgus antipodarum*), zebra mussel (*Dreissena polymorpha*)and quagga mussel (*Dreissena bugensis*)veligers.	No				
	Plants: water meal	No				
	Other Organisms Bacteria, parasites and pathogens of concern to both human health and aquatic organisms; specifically *Escherichia coli* (*E. coli*) and hepatitis.	No				

1 **Tasks** (From Step 2)	2 **Potential Non-Targets** (From Step 3)	3 **Risk Assessment** Are any non-targets significant? Yes or No	4 **Justification** Justify your answer in Column 3	5 **Control** What Control Measures Can be applied during this task to reduce the risk of non-targets?	6 **CCP** Is this task a CCP? Yes or No	7 **Justification** Justify your answer in column 6
Task #3 Title: Processing and handling the shipment.	**Vertebrates:** Small fish of various species	Yes	Once samples are on-station at CERC, the chance for non-target species to be transferred to an environment where they can spread or thrive is highly likely.	If samples are not processed immediately, the cooler and its entire contents should be placed in the walk-in freezer. Once removed from the freezer, the following procedures must be followed. If freezing is not advised due to risk sample containers breaking (a potential risk with very wet sediments in glass jars), then the outside of the cooler should be decontaminated in the quarantine room using a 2% Virkon® solution and then the cooler and its entire contents can be placed in the walk-in cooler. Start by preparing the sample processing table in the quarantine room. Clean off sufficient space and place bench paper on the work area. Carry the cooler directly from the loading dock into the quarantine room. Gloves should be worn while handling the shipment. Sample bottles should be removed from the cooler, dried off and sprayed down with a 2% Virkon® solution and allowed to sit for ten minutes before it is rinsed and wiped dry. Excess water in the cooler should be poured into a bucket dumping station. Excess sediment should be removed with gloved hands and paper towels, placed in a sealed ziplock bag and placed in the trash can. The cooler should then be sprayed with 2% Virkon® and allowed to sit for ten minutes before it is rinsed and wiped dry. The samples can either be repacked in the same cooler or transferred to a clean container and then removed from the room. Bench paper should be rolled up and placed into the trash can. All work surfaces should be cleaned with Virkon® spray and paper towels.	Yes	This is a critical control point because this is the point where the transfer of a non-target species is most likely to occur. Loose sediments or excess water in the cooler has the highest risk of introducing a non-target species to CERC.
	Invertebrates: Numerous invertebrates (worms, crayfish, leeches, barnacles) but specifically New Zealand Mud Snails (*Potamopyrgus antipodarum*), zebra mussel (*Dreissena polymorpha*)and quagga mussel (*Dreissena bugensis*)veligers.	Yes				
	Plants: water meal	Yes				
	Other Organisms Bacteria, parasites and pathogens of concern to both human health and aquatic organisms; specifically *Escherichia coli* (*E. coli*) and hepatitis.	Yes				

1 **Tasks** (From Step 2)	2 **Potential Non-Targets** (From Step 3)	3 **Risk Assessment** Are any non-targets significant? Yes or No	4 **Justification** Justify your answer in Column 3	5 **Control** What Control Measures can be applied during this task to reduce the risk of non-targets?	6 **CCP** Is this task a CCP? Yes or No	7 **Justification** Justify your answer in column 6
Task #4 Title: Storage of the samples	**Vertebrates:** Small fish of various species	No	Risk is low for transferring non-target species because samples and storage cooler have been cleaned thoroughly before storage. Storage of samples in closed bottles in either a cooler or freezer does not allow for non-target species to remain viable.	N/A	No	There are no significant non-targets during this task.
	Invertebrates: Numerous invertebrates (worms, crayfish, leeches, barnacles) but specifically New Zealand Mud Snails (*Potamopyrgus antipodarum*), zebra mussel (*Dreissena polymorpha*)and quagga mussel (*Dreissena bugensis*)veligers.	No		N/A		
	Plants: water meal	No		N/A		
	Other Organisms Bacteria, parasites and pathogens of concern to both human health and aquatic organisms; specifically *Escherichia coli* (*E. coli*) and hepatitis.	No		N/A		

1 **Tasks** (From Step 2)	2 **Potential Non-Targets** (From Step 3)	3 **Risk Assessment** Are any non-targets significant? Yes or No	4 **Justification** Justify your answer in Column 3	5 **Control** What Control Measures Can be applied during this task to reduce the risk of non-targets?	6 **CCP** Is this task a CCP? Yes or No	7 **Justification** Justify your answer in column 6
Task #5 Title: Processing and analyzing the samples.	**Vertebrates:** Small fish of various species	No	The risk is low for transferring non-target species to CERC during this task. If samples have not previously been frozen or stored in an anaerobic environment for a period of time, they are processed using strict controls to minimize the risk for release of any non-target organisms.	Sediment samples can be placed in a sealed ziplock bag and disposed of in the trash following a period of time after the final publication of data as agreed upon by CERC and its collaborator. For samples that cannot be disposed of (e.g., NRDAR projects), arrangements should be made for long-term storage at CERC or another facility during the planning stages of the project.	No	There are no significant non-targets during this task.
	Invertebrates: Numerous invertebrates (worms, crayfish, leeches, barnacles) but specifically New Zealand Mud Snails (*Potamopyrgus antipodarum*), zebra mussel (*Dreissena polymorpha*)and quagga mussel (*Dreissena bugensis*)veligers.	No				
	Plants: water meal	No				
	Other Organisms Bacteria, parasites and pathogens of concern to both human health and aquatic organisms; specifically *Escherichia coli* (*E. coli*) and hepatitis.	No				

HACCP Step 5 – Non-Target Risk Action Plan (NTRAP)

(Use this form for any "Yes" from Column 6 of HACCP Step 4 - Non-Target Analysis Worksheet)
One page for each Critical Control Point

Mangement Objective From Step 1	Import field-collected soil and sediment samples to the Columbia Environmental Research Center (CERC) for analysis of organic and inorganic compounds that may negatively influence ecosystems. Provide this information to management and regulatory agencies.			
Critical Control Point:	**Task #**	3	**Title:**	Processing and handling the shipment.
Significant Non-Target(s) (Step 4, Column 3)	Small fish of various species, numerous small invertebrates (worms, crayfish, leeches) but specifically New Zealand Mud Snails, zebra and quagga mussel veligers, water meal, bacteria, parasites and pathogens of concern to both human health and aquatic organisms; specifically *E. coli*), hepatitis, Aeromonas/Pseudomonas, *Batrachochytrium dendrobatidis* (chytrid fungus).			
Control Measure(s) (Step 4, Column 5)	Start by preparing the sample processing table in the quarantine room. Clean off sufficient space and place bench paper on the work area. Carry the cooler directly from the loading dock into the quarantine room. Gloves should be worn while handling the shipment. Sample bottles should be removed from the cooler, dried off and sprayed down with 2% Virkon® and allowed to sit for ten minutes before it is rinsed and wiped dry. Excess water in the cooler should be poured into a bucket dumping station. Excess sediment should be removed with gloved hands and paper towels, put in a sealed ziplock bag and placed in the trash can. The cooler should then be sprayed with 2% Virkon® and allowed to sit for ten minutes before it is rinsed and wiped dry. The samples can either be repacked in the same cooler or transferred to a clean container and then removed from the room. Bench paper should be rolled up and placed into the trash can. All work surfaces should be cleaned with Virkon® spray and paper towels.			
Precribed ranges, limits, or citeria for control measure(s): (PRLC)	Sample bottles and cooler should be sprayed or wiped with 2% Virkon® for 10 minutes before rinsing or drying. Care must be taken to not damage sample labels. Coolers should be visually inspected after cleaning so there is no visible mud or sediment remaining outside or inside the cooler.			
Monitoring the Control Measure(s)	**Who?**	Chemistry technicians		
	How?	Make sure all surfaces have been cleaned and sprayed with 2% Virkon and allowed to sit for ten minutes.		
	Where?	Quarantine room		
	How often?	When receiving a shipment of soil or sediment samples.		

Corrective Action(s) if Control Measures Fail (or PRLC cannot be met)	Repeat control measures from task 3. Remove all visible dirt, sediments and water from cooler and spray with 2% Virkon®.		
Supporting Documents *(For example, Management Plan, Checklist, Decontamination Techniques, SOPs, Scientific Journal Articles, etc.)*			
Development Team Members	**James Candrl and David Alvarez**		
Date Developed:	**11/7/14**	**Date(s) Reviewed:**	**12/29/14 Biosecurity Committee**

***all fields in grey are required**

CERC Passive Samplers HACCP final Plan

HACCP Step 1 – Activity Description

Management Objective & Contact Information	
HACCP Plan Title: Importation of passive samplers of organic and inorganic chemicals that were deployed in various aquatic environments.	
Management Objective: Import field-collected passive sample devices to the Columbia Environmental Research Center (CERC) for analysis of toxic organic and inorganic compounds. Provide this information to resource managers and regulatory agencies concerned about negative impacts on ecosystem health.	Contact Person: David Alvarez Phone: 573-441-2970 Email: dalvarez@usgs.gov

Activity Description

i.e. Who; What; Where; When; How; Why

Who: Environmental Chemistry Branch
What: Receiving passive sample devices for analysis of organic and inorganic compounds
Where: CERC loading dock and quarantine room
When: Throughout the year
How: Passive sampling devices are received from collaborative partners via shipping carriers (Fedex , UPS, etc.) or collected by CERC personnel and transported to the lab and unloaded on the back loading dock. Cooler is taken to the quarantine room, opened up; an inventory of the sample containers is taken and if needed, samplers and coolers are cleaned at this time. Samplers are then typically stored in a walk-in freezer for later analysis.
Why: To provide scientific data on the presence and/or concentrations of chemical constituents to collaborative partners such as state and federal agencies.

HACCP Step 2 – Activity Flow Chart

Outline Sequential Tasks of Activity

Task 1	Title: Coordination of sampling and shipping logistics.
	Description: Designated person coordinates with the partner agency to ensure that samples are collected and stored properly according to CERC Standard Operating Procedures (SOPs) and Chain of Custody (COC) forms if necessary. Informs staff members of approximate arrival time, intended purpose of the samples and designates appropriate staff to handle samples. Information related to potential biological non-targets at the sampling site and sample receiving are discussed.

Task 2	Title: Shipping the samples and receiving of the shipment at CERC.
	Description: Samples are packaged in a cooler by field crews and shipped by a shipping carrier. Upon arrival at CERC, Samples are off-loaded by shipping carrier onto the back loading dock or occasionally at the front desk. Front desk notifies the recipient that the shipment has arrived.

Task 3	Title: Processing and handling the shipment.
	Description: Cooler is opened up and an inventory of the sample containers is taken. If any of the shipping cans containing the samplers are damaged or open, the passive samplers will be transferred to a new can. Any residual water or sediment on the inside or outside of the cooler is removed, and discarded/treated properly. Both the sample cans and the cooler is cleaned thoroughly inside and out.

Task 4	Title: Repacking and storage of the samples
	Description: Sample devices are either repacked in the cooler or placed in separate storage containers (boxes, tubs, freezer cages, etc.) and taken to the proper location for storage or direct processing of the samples.

Task 5	Title: Processing and analyzing the samples.
	Description: Samplers are processed in the lab according to the appropriate standard operating procedure. All passive samplers will be consumed during the processing. Sampler materials, such as the membranes, which remain following extraction, will be disposed of in the trash. Sampler hardware (metal and plastic components) will be placed in buckets prior to cleaning with acid, soapy water, and organic solvents.

HACCP Step 3 – Identify Potential Non-Targets

Non-Targets That May Potentially Be Moved/Introduced
Vertebrates: Small fish of various species
Invertebrates: Numerous invertebrates (worms, crayfish, leeches, barnacles, crabs) could be potentially be transferred but specifically New Zealand Mud Snails (*Potamopyrgus antipodarum*), zebra mussel (*Dreissena polymorpha*) and quagga mussel (*Dreissena bugensis*)veligers.
Plants: Water meal and algae
Other Organisms (pathogens, parasites, etc.) Bacteria, parasites and pathogens of concern to both human health and aquatic organisms; specifically *Escherichia coli* (*E. coli*) and hepatitis.

1 **Tasks** (From Step 2)	2 **Potential Non-Targets** (From Step 3)	3 **Risk Assessment** Are any non-targets significant? Yes or No	4 **Justification** Justify your answer in Column 3	5 **Control** What Control Measures Can be applied during this task to reduce the risk of non-targets?	6 **CCP** Is this task a CCP? Yes or No	7 **Justification** Justify your answer in column 6
Task #1 Title: Coordination of sampling and shipping logistics.	**Vertebrates:** Small fish of various species	No	There is no risk of transferring non-target species because the samplers have not been shipped yet.	N/A	No	There are no significant non-targets during this task
	Invertebrates: Numerous invertebrates (worms, crayfish, leeches, barnacles, crabs) but specifically New Zealand Mud Snails (*Potamopyrgus antipodarum*), zebra mussel (*Dreissena polymorpha*)and quagga mussel (*Dreissena bugensis*)veligers.	No				
	Plants: water meal and algae	No				
	Other Organisms Bacteria, parasites and pathogens of concern to both human health and aquatic organisms; specifically *Escherichia coli* (*E. coli*) and hepatitis.	No				

1 **Tasks** (From Step 2)	2 **Potential Non-Targets** (From Step 3)	3 **Risk Assessment** Are any non-targets significant? Yes or No	4 **Justification** Justify your answer in Column 3	5 **Control** What Control Measures Can be applied during this task to reduce the risk of non-targets?	6 **CCP** Is this task a CCP? Yes or No	7 **Justification** Justify your answer in column 6
Task #2 Title: Receiving of the shipment at CERC.	**Vertebrates:** Small fish of various species	No	Shipping trucks, warehouses and CERC loading dock are dry, so environment for aquatic species to survive and spread is not likely.	N/A	No	There are no significant non-targets during this task
	Invertebrates: Numerous invertebrates (worms, crayfish, leeches, barnacles, crabs) but specifically New Zealand Mud Snails (*Potamopyrgus antipodarum*), zebra mussel (*Dreissena polymorpha*)and quagga mussel (*Dreissena bugensis*)veligers.	No				
	Plants: water meal and algae	No				
	Other Organisms Bacteria, parasites and pathogens of concern to both human health and aquatic organisms; specifically *Escherichia coli* (*E. coli*) and hepatitis.	No				

1 **Tasks** (From Step 2)	2 **Potential Non-Targets** (From Step 3)	3 **Risk Assessment** Are any non-targets significant? Yes or No	4 **Justification** Justify your answer in Column 3	5 **Control** What Control Measures Can be applied during this task to reduce the risk of non-targets?	6 **CCP** Is this task a CCP? Yes or No	7 **Justification** Justify your answer in column 6
Task #3 Title: Processing and handling the shipment.	**Vertebrates:** Small fish of various species	Yes	Once samplers are on-station at CERC, the chance for non-target species to be transferred to an environment where they can spread or thrive is highly likely.	If samplers are not processed immediately, the cooler and its entire contents should be placed in the walk-in freezer. Once removed from the freezer, the following procedures must be followed: Start by preparing the sample processing table in the quarantine room. Clean off sufficient space and place bench paper on the work area. Carry the cooler directly from the loading dock into the quarantine room. Gloves should be worn while handling the shipment. Sample containers should be removed from the cooler, dried off and sprayed down with a 2% Virkon® solution and allowed to sit for ten minutes before it is rinsed and wiped dry. Excess water in the cooler should be poured into a bucket dumping station. Excess sediment should be removed with gloved hands and paper towels, placed in a sealed ziplock bag and placed in the trash can. The cooler should then be sprayed with 2% Virkon® and allowed to sit for ten minutes before it is rinsed and wiped dry. The samples can either be repacked in the same cooler or transferred to a clean container and then removed from the room. Bench paper should be rolled up and placed into the trash can. All work surfaces should be cleaned with Virkon® spray and paper towels.	Yes	This is a critical control point because this is the point where the transfer of a non-target species is most likely to occur. Loose sediments or excess water in the cooler has the highest risk of introducing a non-target species to CERC.
	Invertebrates: Numerous invertebrates (worms, crayfish, leeches, barnacles, crabs) but specifically New Zealand Mud Snails (*Potamopyrgus antipodarum*), zebra mussel (*Dreissena polymorpha*)and quagga mussel (*Dreissena bugensis*)veligers.	Yes				
	Plants: water meal and algae	Yes				
	Other Organisms Bacteria, parasites and pathogens of concern to both human health and aquatic organisms; specifically *Escherichia coli* (*E. coli*) and hepatitis.	Yes				

1 **Tasks** (From Step 2)	2 **Potential Non-Targets** (From Step 3)	3 **Risk Assessment** Are any non-targets significant? Yes or No	4 **Justification** Justify your answer in Column 3	5 **Control** What Control Measures can be applied during this task to reduce the risk of non-targets?	6 **CCP** Is this task a CCP? Yes or No	7 **Justification** Justify your answer in column 6
Task #4 Title: Storage of the samples	**Vertebrates:** Small fish of various species	No	Risk is low for transferring non-target species because passive sampler containers and storage cooler have been cleaned thoroughly before storage. Storage of samples in closed containers in either a cooler or freezer does not allow for non-target species the potential to escape.	N/A	No	There are no significant non-targets during this task.
	Invertebrates: Numerous invertebrates (worms, crayfish, leeches, barnacles, crabs) but specifically New Zealand Mud Snails (*Potamopyrgus antipodarum*), zebra mussel (*Dreissena polymorpha*)and quagga mussel (*Dreissena bugensis*)veligers.	No		N/A		
	Plants: water meal and algae	No		N/A		
	Other Organisms Bacteria, parasites and pathogens of concern to both human health and aquatic organisms; specifically *Escherichia coli* (*E. coli*) and hepatitis.	No		N/A		

1 **Tasks** (From Step 2)	2 **Potential Non-Targets** (From Step 3)	3 **Risk Assessment** Are any non-targets significant? Yes or No	4 **Justification** Justify your answer in Column 3	5 **Control** What Control Measures Can be applied during this task to reduce the risk of non-targets?	6 **CCP** Is this task a CCP? Yes or No	7 **Justification** Justify your answer in column 6
Task #5 Title: Processing and analyzing the samples.	**Vertebrates:** Small fish of various species	No	The risk is low for transferring non-target species to CERC during this task. If samplers have not previously been frozen or stored in an anaerobic environment for a period of time, they are processed using strict controls to minimize the risk for release of any non-target organisms.	Any remaining materials (membranes) from the passive samplers following extraction are to be disposed of in the trash. Deployment hardware is placed in buckets or tubs to dry, after which they are cleaned using acids and solvents according to laboratory SOP P.585.	No	There are no significant non-targets during this task.
	Invertebrates: Numerous invertebrates (worms, crayfish, leeches, barnacles, crabs) but specifically New Zealand Mud Snails (*Potamopyrgus antipodarum*), zebra mussel (*Dreissena polymorpha*)and quagga mussel (*Dreissena bugensis*)veligers.	No				
	Plants: water meal and algae	No				
	Other Organisms Bacteria, parasites and pathogens of concern to both human health and aquatic organisms; specifically *Escherichia coli* (*E. coli*) and hepatitis.	No				

HACCP Step 5 – Non-Target Risk Action Plan (NTRAP)

(Use this form for any "Yes" from Column 6 of HACCP Step 4 - Non-Target Analysis Worksheet)
One page for each Critical Control Point

Mangement Objective From Step 1	Import passive sampler devices to the Columbia Environmental Research Center (CERC) for analysis of organic and inorganic compounds that may negatively influence ecosystems. Provide this information to management and regulatory agencies.			
Critical Control Point:	**Task #**	3	**Title:**	Processing and handling the shipment.
Significant Non-Target(s) (Step 4, Column 3)	Small fish of various species, numerous small invertebrates (worms, crayfish, leeches, crabs) but specifically New Zealand Mud Snails, zebra and quagga mussel veligers, water meal and algae, numerous pathogens of concern to both human health (*E. coli,hepatitis*) and aquatic species.			
Control Measure(s) (Step 4, Column 5)	Start by preparing the sample processing table in the quarantine room. Clean off sufficient space and place bench paper on the work area. Carry the cooler directly from the loading dock into the quarantine room. Gloves should be worn while handling the shipment. Passive sampler containers should be removed from the cooler, dried off and sprayed down with 2% Virkon® and allowed to sit for ten minutes before it is rinsed and wiped dry. Excess water in the cooler should be poured into a bucket dumping station. Excess sediment should be removed with gloved hands and paper towels, put in a sealed ziplock bag and placed in the trash can. The cooler should then be sprayed with 2% Virkon® and allowed to sit for ten minutes before it is rinsed and wiped dry. The sample containers can either be repacked in the same cooler or transferred to a clean container and then removed from the room. Bench paper should be rolled up and placed into the trash can. All work surfaces should be cleaned with Virkon® spray and paper towels.			
Precribed ranges, limits, or citeria for control measure(s): (PRLC)	Sampler device containers and cooler should be sprayed or wiped with 2% Virkon® for 10 minutes before rinsing or drying. Care must be taken to not damage sample labels. Coolers should be visually inspected after cleaning so there is no visible mud or sediment remaining outside or inside the cooler.			
Monitoring the Control Measure(s)	**Who?**	Chemistry technicians		
	How?	Make sure all surfaces have been cleaned and sprayed with 2% Virkon and allowed to sit for ten minutes.		
	Where?	Quarantine room		
	How often?	When receiving a shipment of passive sampler devices.		
Corrective Action(s) if Control Measures Fail (or PRLC cannot	Repeat control measures from task 3. Remove all visible dirt, sediments and water from cooler and spray with 2% Virkon®.			

be met)			
Supporting Documents *(For example, Management Plan, Checklist, Decontamination Techniques, SOPs, Scientific Journal Articles, etc.)*			
Development Team Members	**James Candrl and David Alvarez**		
Date Developed:	**11/7/14**	**Date(s) Reviewed:**	**12/29/14 Biosecurity Committee**

***all fields in grey are required**

CERC Field Collection of Aquatic Organisms and Biological, Sediment, and Water Samples HACCP Plan (DRAFT)

HACCP Step 1 – Activity Description

Management Objective & Contact Information	
HACCP Plan Title: Field sampling for aquatic organisms (crayfish); Field collection of biological, sediment, and water samples for toxic inorganic chemical analysis	
Management Objective: Field sampling for aquatic organisms (crayfish); and field-collection of biological, sediment, and water samples for inorganic compounds. Provide this information to resource managers and regulatory agencies concerned about negative impacts on ecosystem health.	Contact Person: Ann L. Allert Phone: 573-876-1903 Email: aallert@usgs.gov

Activity Description

i.e. Who; What; Where; When; How; Why

Who: Ecology Branch, Ecological Restoration Branch
What: Field collection of crayfish for estimates of density and an in-situ toxicity test, and field collection of biologic samples biological, sediment, and water samples for toxic inorganic chemical analysis
Where: Little St. Francis River and tributaries, St. Francis and Madison County, Missouri, USA; on-site processing of samples at Farmington, MO, USA
When: March to August 2015 (conditions permitting)
How: Field collection of **crayfish** for estimates of density will be done using a 1-m^2 quadrat or kick-seine sampler. Field collection of **crayfish for in-situ toxicity test** will be done using hand-nets or seines. Crayfish not used as voucher specimens or for inorganic analyses (metals) will be returned to the stream where they were collected. Crayfish not used in in-situ toxicity tests will be euthanized.
Field collection of samples for inorganic analyses: A subsample of crayfish (n =3 per site; 3–5 crayfish per sample) will be placed in HDPE jars after they have been identified to species and carapace length measured. **Sediment** samples (n =3 per site) will be taken using a plastic scoop, 2-mm filtration bucket, and pre-cleaned jars. Subsurface grab samples of **water** (n =3 per site) will be taken using a pre-cleaned 1-gal container. Water will be filtered or placed in pre-cleaned sample containers depending on analysis. Equipment used to process samples will be rinsed with reverse-osmosis water and dried. Water used to rinse equipment will be put into the sanitary waste system. **Pore water** will be collected from sediments and placed into pre-cleaned jars. **Macroinvertebrates, leaves, and crayfish** will be taken from cages and each placed into pre-cleaned jars (n =3 per site) on two sampling dates.
Why: To provide scientific data on the effects of trace metals on crayfish in the Little St. Francis River with cooperation from the Missouri Department of Conservation, in support of a National Damage Assessment (NRDAR) for US Fish and Wildlife Service.

HACCP Step 2 – Activity Flow Chart

Outline Sequential Tasks of Activity

Task 1	Title: Coordination of sampling and shipping logistics.
	Description: Designated person coordinates with the CERC personnel or partner agency to ensure that crayfish are collected according to CERC Standard Operating Procedures (SOPs) and Animal Welfare Plan (AWP). All collections will be implemented in the spirit and intent of the policies and regulations that assure humane and ethical treatment of research animals. Informs staff members of approximate arrival time, intended purpose of the crayfish and designates appropriate staff to handle samples. Designated person coordinates with the CERC personnel or partner agency to ensure that samples are collected properly according to CERC Standard Operating Procedures (SOPs); processed according to CERC HAACPs for the Collection of Biological, Sediment, and Water Samples, and for Passive Samplers; and stored with Chain of Custody (COC) forms if necessary. Informs staff members of approximate arrival time, intended purpose of the samples and designates appropriate staff to handle samples. Information related to potential biological non-targets at the sampling site and sample receiving are discussed.

Task 2	Title: Shipping the samples and receiving of the shipment at CERC.
	Description: Crayfish are packaged in cooler by field crews and delivered by field crews to CERC. Upon arrival at CERC, coolers with crayfish are off-loaded onto the back loading dock. Samples are processed and packaged in a cooler by field crews and delivered by field crews to CERC. Upon arrival at CERC, samples are off-loaded onto the back loading dock.

Task 3	Title: Processing and handling the shipment.
	Description: Coolers with crayfish are opened on loading deck and **CERCs Protocols for the Transfer of Crayfish** will be followed to transfer crayfish into CERCs Quarantine Room. CERC LIVE ORGANISM TRANSFER REQUEST (LOTR) forms will be transferred to Biosecurity Committee. Coolers with samples will be opened and an inventory of the samples will be confirmed using COC forms. Samples will be processed according to CERC HAACPs for the Collection of Biological, Sediment, and Water Samples, and for Passive Samplers.

Task 4	Title: Repacking and storage of the samples
	Description: Crayfish are placed in designated tanks with secured tops and effluent treatment (chlorine and/or UV light) in Quarantine Room. Culture of crayfish will follow Rules for CERC Quarantine Room. Samples are either repacked in the cooler or placed in separate storage containers (boxes, tubs, freezer cages, etc.) and taken to the proper location for storage or direct processing of the samples.

Task 5	Title: Processing and analyzing the samples.
	Crayfish will be maintained in Quarantine Room until use in in-situ field study; all rules and regulations will be followed while entering, working in, and leaving Quarantine Room. Description: Samples are processed in the lab according to the appropriate standard operating procedure and HACCPs. Excess sample will be disposed of according to CERC's hazardous substances and biosecurity guidelines.

HACCP Step 3 – Identify Potential Non-Targets

Non-Targets That May Potentially Be Moved/Introduced
Vertebrates: USGS NAS database lists the following organisms non-native invasives in the St. Francis Drainage*:* Cyprinidae, *Cyprinus carpio***,** Common carp; Centrarchidae (established Little St. Francis River in Madison County), *Ambloplites rupestris*, rock bass (established Little St. Francis River in Madison County), Cyprinidae, *Notropis buccatus*, silverjaw minnow (collected St. Francis River in Madison County).
Invertebrates: USGS NAS database lists the following organisms non-native invasives in the St. Francis Drainage*:* *Orconectes hylas* (Crustacean Decapoda; woodland crayfish; collected in St. Francis River and tributaries in St. Francis and Madison County); *Orconectes harrisonii* (Crustacean Decapoda; belted crayfish; collected in St. Francis River in St. Francis County); *Craspedacusta sowerbyi* (Coelenterates-Hydrozoan; freshwater jelly fish; collected in Cedar Lake and Lac Benet near Bonne Terre in St. Francis County); *Daphnia lumholtzi* (Crustaceans-Cladocerans; waterflea); Mollusks-Bivales, Corbiculidae, *Corbicula fluminea*; Asian clam (collected St. Francis River in Madison County).
Plants: Filamentous green algae (Chlorophyta/Charophyta) and potential other aquatic vegetation that is distributed Statewide in Missouri. None is listed for Little St. Francis River, but staff should be aware of the potential for their presence.
Other Organisms (pathogens, parasites, etc.) Bacteria, parasites and pathogens of concern to both human health and aquatic organisms; specifically *Escherichia coli* (*E. coli*), hepatitis, Aeromonas/Pseudomonas, *Batrachochytrium dendrobatidis* (chytrid fungus).

1 **Tasks** (From Step 2)	2 **Potential Non-Targets** (From Step 3)	3 **Risk Assessment** Are any non-targets significant? Yes or No	4 **Justification** Justify your answer in Column 3	5 **Control** What Control Measures Can be applied during this task to reduce the risk of non-targets?	6 **CCP** Is this task a CCP? Yes or No	7 **Justification** Justify your answer in column 6
Task #1 Title: Coordination of sampling; and shipping logistics.	**Vertebrates:** Common carp; rock bass silverjaw minnow	No	Fish can be identified and euthanized prior to leaving site.	Bleach; MS-222; ice; Virkon® spray and paper towels	Yes	Preventative measures should be taken before leaving field sites.
	Invertebrates: several	No	When possible, invertebrates will be identified, removed, and/or euthanized prior to leaving sites. Gear will be cleaned between sites to prevent spread of organisms too small to visible identify. Gear will be cleaned according to Missouri Department of Conservation Cleaning and Disinfecting Gear and Equipment Protocols. Containers will be cleaned on-site according to CERC HAACPs for the Collection of Biological, Sediment, and Water Samples, and for Passive Samplers.	Bleach; MS-222; ice; Virkon® spray and paper towels		
	Plants: several	No	Gear will be cleaned between sites to prevent spread of organisms too small to visible identify. Gear will be cleaned according to Missouri Department of Conservation Cleaning and Disinfecting Gear and Equipment Protocols. Containers will be cleaned according to CERC HAACPs for the Collection of Biological, Sediment, and Water Samples, and for Passive Samplers.	Bleach; MS-222; ice; Virkon® spray and paper towels		
	Other Organisms Bacteria and pathogens of concern to both human health and aquatic organisms.	No	Gear will be cleaned between sites to prevent spread of organisms too small to visible identify. Gear will be cleaned according to Missouri Department of Conservation Cleaning and Disinfecting Gear and Equipment Protocols. CERC Protocols for the Receipt and Transfer of Crayfish will be followed. Containers will be cleaned according to CERC HAACPs for the Collection of Biological, Sediment, and Water Samples, and for Passive Samplers.	Salt; bleach; MS-222; ice; UV, Virkon® spray and paper towels		

1 **Tasks** (From Step 2)	2 **Potential Non-Targets** (From Step 3)	3 **Risk Assessment** Are any non-targets significant? Yes or No	4 **Justification** Justify your answer in Column 3	5 **Control** What Control Measures Can be applied during this task to reduce the risk of non-targets?	6 **CCP** Is this task a CCP? Yes or No	7 **Justification** Justify your answer in column 6
Task #2 Title: Receiving of crayfish and sample shipment at CERC.	**Vertebrates:** None	No	CERC Protocols for the Receipt and Transfer of Crayfish will be followed There is a very low risk of transferring non-target species because samples are contained in coolers and kept sealed and because containers will be cleaned on-site according to CERC HAACPs for the Collection of Biological, Sediment, and Water Samples, and for Passive Samplers.	N/A	No	There are no significant non-targets during this task.
	Invertebrates: several	Yes		Salt, bleach, UV, temperature, Virkon® spray and paper towels		
	Plants: None	No		N/A		
	Other Organisms Bacteria and pathogens of concern to both human health and aquatic organisms.	Yes		Bleach; MS-222; ice; UV, Virkon® spray and paper towels		

1 **Tasks** (From Step 2)	2 **Potential Non-Targets** (From Step 3)	3 **Risk Assessment** Are any non-targets significant? Yes or No	4 **Justification** Justify your answer in Column 3	5 **Control** What Control Measures Can be applied during this task to reduce the risk of non-targets?	6 **CCP** Is this task a CCP? Yes or No	7 **Justification** Justify your answer in column 6
Task #3 Title: Processing crayfish and handling the sample shipment.	**Vertebrates:** None	No	Once samples are on-station at CERC, the chance for non-target species to be transferred to an environment where they can spread or thrive is highly likely.	Crayfish should be processed immediately following CERC Protocols for the Receipt and Transfer of Crayfish. If samples are not processed immediately, the cooler and its entire contents should be placed in the walk-in freezer. Once removed from the freezer, the following procedures must be followed: CERC personal will follow CERC HAACPs for Biological, Sediment, and Water Samples, and for Passive Samplers upon return to CERC. Storage of samples in freezer should kill invertebrates.	Yes	This is a critical control point because this is the point where the transfer of a non-target species is most likely to occur. Surfaces that tissue samples are processed on have the highest risk of introducing a non-target species to CERC.
	Invertebrates: several	Yes				
	Plants: None	No				
	Other Organisms Bacteria and pathogens of concern to both human health and aquatic organisms.	Yes				

1 **Tasks** (From Step 2)	2 **Potential Non-Targets** (From Step 3)	3 **Risk Assessment** Are any non-targets significant? Yes or No	4 **Justification** Justify your answer in Column 3	5 **Control** What Control Measures can be applied during this task to reduce the risk of non-targets?	6 **CCP** Is this task a CCP? Yes or No	7 **Justification** Justify your answer in column 6
Task #4 Title: Culture of crayfish and storage of the samples	**Vertebrates:** None	No	Culture of crayfish will follow Rules for CERC Quarantine Room. Risk is low for transferring non-target species out of culture tank or through effluent of culture tank due to control measures to keep crayfish in tanks (tops) or treat effluent (UV/chlorine). All equipment and surfaces will be sprayed with 2% Virkon® and allowed to sit for ten minutes before it is rinsed and wiped dry. All wasted will be placed into the trash can then frozen before disposal. All materials including feces removed from tanks holding crayfish will be frozen before disposal. Risk is low for transferring non-target species because samples and storage cooler have been cleaned thoroughly before storage. Storage of samples in sealed containers in either a cooler or freezer minimizes any potential transfer of pathogens.	N/A	No	There are no significant non-targets during this task.
	Invertebrates: several	No				
	Plants: None	No				
	Other Organisms Bacteria and pathogens of concern to both human health and aquatic organisms.	No				

1	2	3 **Risk**	4	5 **Control**	6	7

Tasks (From Step 2)	**Potential Non-Targets** (From Step 3)	**Assessment** Are any non-targets significant? Yes or No	**Justification** Justify your answer in Column 3	What Control Measures Can be applied during this task to reduce the risk of non-targets?	**CCP** Is this task a CCP? Yes or No	**Justification** Justify your answer in column 6
Task #5 Title: Processing and analyzing crayfish and the samples.	**Vertebrates:** None	No	If work area is not thoroughly disinfected after processing the samples, the risk of transferring pathogens throughout the lab is present.	All processing of crayfish will occur in the Quarantine Room and care should be given to not working over open drains. Constant checks around coolers should be made to ensure no crayfish have fallen to floor or escaped coolers. All equipment and surfaces will be sprayed with 2% Virkon® and allowed to sit for ten minutes before it is rinsed and wiped dry. All wasted will be placed into the trash can then be frozen before disposal. During the processing of samples, Best Lab Practices (BLP's) should be used at all times including wearing gloves while handling the samples. Upon completion of sample processing, all work and floor surfaces and equipment used during the processing must be sprayed down with a 2% Virkon® Aquatic or 2% chlorine solution and allowed to sit for ten minutes before surfaces are rinsed. Excess tissue samples can be placed in a bag and disposed of in the trash following a period of time after the final publication of data as agreed upon by CERC and its collaborator. For samples that cannot be disposed of (e.g., NRDAR projects), arrangements should be made for long-term storage at CERC or another facility during the planning stages of the project.	Yes	Samples will be exposed and handled on work surfaces potentially allowing pathogens to be transferred throughout the center and potentially infecting existing cultures or research projects.
	Invertebrates: several	No				
	Plants: None	No				
	Other Organisms Bacteria and pathogens of concern to both human health and aquatic organisms.	Yes				

HACCP Step 5 – Non-Target Risk Action Plan (NTRAP)

(Use this form for any "Yes" from Column 6 of HACCP Step 4 - Non-Target Analysis Worksheet)
One page for each Critical Control Point

Mangement Objective From Step 1	Import field-collected crayfish and field-collected non-living biologic, sediment and water samples and passive samplers to the Columbia Environmental Research Center (CERC) for analysis of toxic inorganic compounds that may negatively influence ecosystems and culture of crayfish for an in-situ toxicity test. Provide this information to resource managers and regulatory agencies.			
Critical Control Point:	**Task #**	3	**Title:**	Processing and handling the shipment.
Significant Non-Target(s) (Step 4, Column 3)	Bacteria, parasites and pathogens of concern to both human health and aquatic organisms; specifically *Escherichia coli* (*E. coli*), hepatitis, Aeromonas/Pseudomonas, *Batrachochytrium dendrobatidis* (chytrid fungus)			
Control Measure(s) (Step 4, Column 5)	Control Measures for the Field: Gear will be cleaned between sites to prevent spread of organisms too small to visible identify. Gear will be cleaned according to Missouri Department of Conservation Cleaning and Disinfecting Gear and Equipment Protocols. Disinfectant Sterilization: The disinfectant of choice is bleach (sodium hypochlorite). Disinfection is accomplished by using a 200-ppm bleach solution to immerse or rinse all field gear and equipment prior to going to another site. Gear and equipment (boots, waders, nets, traps, seines, wetsuits, etc.) will be completely sprayed or immersed in the disinfectant solution for 15 minutes or completely wetted with a bleach solution around 50% if immersing is not possible. Equipment needed include: a long handled brush 5–10 gallon bucket, sprayer. Brush should be used to remove mud, algae, and other debris from boots, waders, etc. It is best to allow the disinfectant solution to air dry on field gear and equipment if possible. If unable to air dry, rinse disinfectant solution with clean water on dry land not in the wetland. The remaining disinfectant solution should be poured on dry land not in water when finished. Desiccation Sterilization: Air drying of gear and equipment can also be used as a preventative for spreading and killing pathogens. Complete air drying, preferably in direct sunlight, at an air temperature of 86°F (30°C) or higher for 4 hours. Drying, as a strategy to sterilize gear and equipment, would have to be long enough to ensure that all water had evaporated. This method is the preferred method for scientific meters (i.e., water quality instruments, XRF). Control Measures for Receipt at CERC: CERC Protocols for the Receipt and Transfer of Crayfish will be followed. Containers will be cleaned according to CERC HAACPs for the Collection of Biological, Sediment, and Water Samples, and for Passive Samplers.			
Precribed ranges, limits, or citeria for control measure(s): (PRLC)	Sample bottles and cooler should be sprayed or wiped with 2% Virkon® for 10 minutes before rinsing or drying. Care must be taken to not damage sample labels. Coolers should be visually inspected after cleaning so there is no visible mud or sediment remaining outside or inside the cooler.			
Monitoring the Control Measure(s)	**Who?**	Ecology and Chemistry technicians		
	How?	Make sure all surfaces and containers have been cleaned and sprayed with 2% Virkon or bleach and allowed to sit for ten minutes.		
	Where?	Quarantine room		
	How often?	When receiving a shipment of crayfish or biological, sediment, water samples or passive sampler .		

Corrective Action(s) if Control Measures Fail (or PRLC cannot be met)	Repeat control measures from task 3. Remove all visible dirt, sediments and water from cooler and spray with 2% Virkon® or bleach.
Supporting Documents *(For example, Management Plan, Checklist, Decontamination Techniques, SOPs, Scientific Journal Articles, etc.)*	

HACCP Step 5 – Non-Target Risk Action Plan (NTRAP)

(Use this form for any "Yes" from Column 6 of HACCP Step 4 - Non-Target Analysis Worksheet)
One page for each Critical Control Point

Mangement Objective From Step 1	Import field-collected crayfish and field-collected non-living biologic, sediment and water samples and passive samplers to the Columbia Environmental Research Center (CERC) for analysis of toxic inorganic compounds that may negatively influence ecosystems. Provide this information to resource managers and regulatory agencies.			
Critical Control Point:	**Task #**	5	**Title:**	Processing and analyzing the samples.
Significant Non-Target(s) (Step 4, Column 3)	Bacteria and pathogens of concern to both human health and aquatic organisms.			
Control Measure(s) (Step 4, Column 5)	All processing of crayfish will occur in the Quarantine Room and care should be given to not working over open drains. Crayfish should be processed following CERC Protocols for the Receipt and Transfer of Crayfish. Constant checks around coolers should be made to ensure no crayfish have fallen to floor or escaped coolers. All equipment and surfaces will be sprayed with 2% Virkon® or bleach and allowed to sit for ten minutes before it is rinsed and wiped dry. All wasted will be placed into the trash can then be frozen before disposal. During the processing of samples, Best Lab Practices (BLP's) should be used at all times including wearing gloves while handling the samples. Upon completion of sample processing, all work and floor surfaces and equipment used during the processing must be sprayed down with a 2% Virkon® Aquatic solution or a 2% chlorine solution and allowed to sit for ten minutes before surfaces are rinsed.			
Precribed ranges, limits, or citeria for control measure(s): (PRLC)	All work surfaces and equipment should be sprayed or wiped with 2% Virkon® or 2% chlorine solution for 10 minutes before rinsing or drying.			
Monitoring the Control Measure(s)	**Who?**	Ecology and Chemistry technicians		
	How?	Make sure all surfaces have been cleaned and sprayed with 2% Virkon or 2% chlorine and allowed to sit for ten minutes.		
	Where?	Grinding room		
	How often?	When receiving a shipment of tissue samples.		
Corrective Action(s) if Control Measures Fail (or PRLC cannot be met)	Repeat control measures from task 5. Remove all visible dirt, sediments and water from cooler and spray with 2% Virkon® or 2% chlorine.			

Supporting Documents
(For example, Management Plan, Checklist, Decontamination Techniques, SOPs, Scientific Journal Articles, etc.)

Development Team Members		**James Candrl and Ann Allert**	
Date Developed:	**05/01/2015**	**Date(s) Reviewed:**	**Biosecurity Committee**

***all fields in grey are required**

Appendix 12: Missouri Department of Conservation Cleaning and Disinfecting Gear and Equipment Protocols

Due to the potential of spreading pathogens, especially amphibian chytrid fungus (*Batrachochytrium dendrobatidas*) and Ranavirus (Iridoviridae), among survey sites, all field gear and equipment should be cleaned and disinfected.

Specific equipment should be cleaned between sampling sites. At minimum any gear and equipment (boots, waders, nets, traps, seines, wetsuits, etc.) that has been in contact with the soil or water should be cleaned and disinfected before, between, and after each wetland (river, pond, pool, etc.) either by use of disinfectant, desiccation, or heat sterilization.

1. **Disinfectant Sterilization:** The disinfectant of choice is bleach (sodium hypochlorite). Disinfection is easily accomplished by putting 4 ounces of bleach (half cup) in one gallon of clean water and using this solution (3% disinfectant solution) to immerse or rinse off all field gear and equipment prior to going to another wetland (river, pond, pool, etc.). Gear and equipment (boots, waders, nets, traps, seines, wetsuits, etc.) should completely be immersed in the disinfectant solution for 15 minutes or completely wetted with a bleach solution around 50% if immersing is not possible. For this disinfecting process, a long handled brush and 5–10 gallon bucket should be considered standard equipment for field investigators. Brush should be used to remove mud, algae, and other debris from boots, waders, etc. Bleach is the disinfectant of choice because it is the least hazardous chemical for disposal, and it is known to decompose rapidly in the environment. It is best to allow the disinfectant solution to air dry on field gear and equipment if possible. If unable to air dry, rinse disinfectant solution with clean water on dry land not in the wetland. The remaining disinfectant solution should be poured on dry land not in water when finished.

2. **Desiccation Sterilization:** Air drying of gear and equipment will also suffice as a preventative for spreading and killing pathogens. Complete air drying, preferably in direct sunlight, at an air temperature of 86°F (30°C) or higher for 4 hours will suffice. Drying, as a strategy to sterilize gear and equipment, would have to be long enough to ensure that all water had evaporated.

3. **Heat Sterilization**: Gear and equipment soaked in hot water will also eliminate pathogens. Heat sterilization at 160°F (71°C) for 20 minutes or 117°F (47 ^{o}C) for 30 minutes will eliminate pathogens.

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