Summary of Program Directive #: MSG-05-03

Subject: Macroinvertebrate sampling to determine if selenium concentrations are exceeding ecological risk guidelines in three wetland areas and the sediment pond at the MMTS.

Directive/Task Changes: Attachment 1 to Program Directive MSG-05-03 specifies the plan for macroinvertebrate sampling to determine if concentrations of selenium in the macroinvertebrates of the wetlands and sediment pond are exceeding ecological risk guidelines for the protection of fish and wildlife species.

Affected Program Documents: Monticello Mill Tailings Operable Unit III Post-Record of Decision Monitoring Plan

Justification: The scope of Program Directive MSG-05-03 is limited to sampling aquatic macroinvertebrates from the three constructed wetlands and the sediment pond based on a meeting held at the site on October 5-6, 2004, with the U.S. Department of Energy, the U.S. Environmental Protection Agency Region VIII, U.S. Fish and Wildlife, and the Utah Department of Environmental Quality. No site upstream of the wetlands that can be considered a control site will be sampled. Emergent, flying macroinvertebrate samples will be collected and archived for analysis at a later date, if necessary.

Effective Date: April 4, 2005

Expiration Date: September 30, 2005
Macroinvertebrate Sampling and Analysis Plan

I. Purpose

Macroinvertebrate samples will be collected in the spring of 2005 at three constructed wetlands and the sediment retention pond (Figure 1 – attached) at the Monticello Mill Tailings Site (MMTS). The collection of macroinvertebrates for selenium analyses is necessary to satisfy the requirements of Section 6.2 of the Monticello Mill Tailings Site Operable Unit III Post-Record of Decision Monitoring Plan (DOE 2004). The concern is that the increasing concentration of selenium in the groundwater that has been observed since completion of millsite remediation will lead to increases in selenium in surface water and sediment that can affect fish and wildlife (particularly avian species) from the consumption of selenium through the food web.

II. Sampling Scope

The Biomonitoring Plan (DOE 2004) requires that macroinvertebrate sampling will be conducted in the second year of biomonitoring and during subsequent years, as warranted. The locations and general approach for macroinvertebrate sampling was discussed October 5-6, 2004 with the Biological Technical Assistance Group (BTAG). Members at the BTAG meeting included representatives from U.S. Department of Energy (DOE), U.S. Environmental Protection Agency (EPA) Region VIII, U.S. Fish and Wildlife Service, and Utah Department of Environmental Quality. The general approach for macroinvertebrate sampling included collecting samples at three constructed wetlands and a sediment pond. Aquatic macroinvertebrate samples would be analyzed for selenium after collection. Emergent, winged macroinvertebrate samples would be collected and archived for analyses (if necessary) at a later date.

III. Background and Need for Sampling

The MMTS, located south of the town of Monticello in southeastern Utah, consisted of a former uranium and vanadium ore-processing mill. In 1989, the site was placed on the National Priorities List under the Comprehensive Environmental Response, Compensation and Liability Act. DOE, EPA Region VIII, and other Federal and state agencies have worked together for the past several years to remediate contaminated soils, surface water and ground water at the MMTS.

Following completion of remediation of Operable Unit III, groundwater, surface water and sediment were monitored for selenium to determine if concentrations may be increasing in three manmade wetlands, and a sedimentation pond (Figure 1), which is located approximately one mile east of the wetlands. The monitoring plan (DOE 2004) specifies the benchmarks for selenium of 4 mg/kg in sediments, and 5 µg/L in surface water. The surface water benchmark has been exceeded and therefore triggered macroinvertebrate sampling for 2005. The sources and concentrations of selenium in groundwater, surface water and sediment are being investigated under Program Directive MSG-04-01, and are scheduled to continue thorough 2006. The need for additional macroinvertebrate sampling after 2005 will be dependent on monitoring results and consultations with BTAG.

Selenium has the ability to accumulate in organisms and the aquatic food chain. Reproductive effects have been observed in fish and wildlife (particularly avian species) that ingest
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Macroinvertebrates with high concentrations of selenium. Guidelines for the dietary threshold for selenium are based upon selenium residues in macroinvertebrates. The no effects level is 3 mg/kg selenium dry weight, and the toxic effects is 7 mg/kg selenium dry weight (Maier and Knight 1994; Lemly 1993 and 1996; Hamilton and Lemly 1999; Beckon et al. 1999; FWS 2004). Past monitoring activities within Montezuma Creek at MMTS found levels of selenium in macroinvertebrates that ranged from approximately 7 to 10 mg/kg in 1995 and 1996 (Peterson et al. 2002). The ecological risk guidelines were considered when setting the benchmarks for surface water, sediment, and macroinvertebrates in the monitoring plan for MMTS (DOE 2004).

The biomonitoring plan states that if analyses of macroinvertebrate samples result in concentrations that exceed 7 mg/kg dry weight, then follow-on work should continue to determine if food chain effects are occurring (DOE 2004). The macroinvertebrate sampling will be done in conjunction with the on-going surface water and sediment sampling (Program Directive MSG-04-01) as well as wildlife surveys (Program Directive MSG 05-01). The results of the aquatic macroinvertebrate samples will be compared to the benchmarks for selenium effects. If the aquatic macroinvertebrate samples exceed 7 mg/kg dry weight benchmark and the wildlife survey indicates that there is a population of birds in the area that would consume the emergent, flying macroinvertebrates, then those samples that have been collected and archived will be analyzed for selenium concentration. In consultation with the BTAG, the results of the macroinvertebrate sampling will be used to determine if bird eggs should be sampled for evidence of toxicity (DOE 2004).

IV. Field Sampling

The purpose of the field sampling effort is to collect macroinvertebrates for selenium analysis to determine if the macroinvertebrates in wetlands 1, 2, and 3 as well as the sediment pond are at levels of concern for selenium. The wetlands will only be sampled using kick nets (Peterson et al. 2002). Other sampling devices for macroinvertebrates are not likely to be effective because of the dense emergent vegetation and shallow waters in the wetlands. The deeper sediment pond will be sampled using both kick nets and artificial substrate samplers (Hester-Dendy samplers). Each replicate sample for selenium analysis will have a minimum mass of 3 g wet weight.

The goal for each sampling location will be to collect three replicate samples. The goal for the sediment pond will be to collect three replicate samples using kick nets and Hester-Dendy samplers. In addition to collecting aquatic macroinvertebrates, light traps at each wetland and the sediment pond will be used to collect emergent, flying macroinvertebrates during the nocturnal period. These samples will be archived for analysis at a later date.

If the goal of collecting a mass of 3 g wet weight per replicate sample can not be achieved, then samples for a location will be composited prior to analysis. It is possible for a sample with a mass of less than 3 g wet weight to be analyzed for selenium at the detection limits necessary. Based on discussions with the analytical laboratory, it may be possible to analyze samples as small as 1 g wet weight. The compromise with a laboratory that conducts analyses on samples with a mass of less than 3 g wet weight is that not all of the laboratory quality control measures may be able to be conducted. The decision to composite the mass collected from all the replicate
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sampling efforts at a location due to lack of adequate mass will depend on input from the analytical laboratory. Those arrangements will be discussed prior to field sampling.

A. Sampling Wetlands 1-3

Each of the three wetlands will be sampled using the same method. Wetlands will be visually divided into three parts for the collection of replicate samples. Flagging will be used to designate the three zones. The three parts will correspond to the flow of water through the wetlands: up-gradient, middle and down-gradient of surface and groundwater flow. The areas where the sample will be collected using the kick nets will be indicated as best as possible on a map. If possible, global positioning system (GPS) measurements will be made at the flags and near the general location where samples were collected.

D-shaped aquatic kick nets with 500 um mesh netting will be used to collect macroinvertebrate samples (Peterson et al. 2002). Each net will be pre-cleaned using a non-phosphate detergent, followed by 2-5% nitric acid wash, and three rinses with laboratory-grade deionized water. The pre-cleaning process is designed to minimize contamination from field equipment prior to use. Nets will be dedicated to each wetland. The same net could be used for replicate sampling within a wetland.

The nets will be used around the perimeter of the wetland and in areas where there is open water between the emergent vegetation. The nets will be worked into the vegetation and along the water sediment interface. If macroinvertebrates are observed on the submerged vegetation, they will also be collected. Field personnel will wear Nitrile gloves during sample collection, and gloves will be changed between sample locations.

Replicate samples will represent a composite of macroinvertebrates. The field samplers will make qualitative notes during the collection of replicate samples at a location. These notes will include information about the functional feeding groups of the macroinvertebrates (Peterson et al. 2002): detritivores (e.g., Tipulidae, Limnephilidae); predators (e.g., Aeshnidae, beetles); and filter feeders (e.g., Hydropychidae, Simuliidae). The field notes will be used to characterize the sample variability in the final report.

B. Sampling the Sediment Pond

The sediment pond will be sampled using both kick nets and Hester-Dendy Multi-Plate samplers. Due to the depth of the sediment pond, only the edges of the pond can be effectively sampled. Kick net samples will be collected using the same method as for collecting in the wetlands. The method for using the Hester-Dendy samplers is described below. Use of both techniques will ensure that sufficient sample masses are collected. In addition, comparing the results from the two sampling techniques will indicate if the preparation of the kick samples was biased by collection method.

The Hester-Dendy samplers are artificial substrate systems that allow water-column sampling of macroinvertebrates. The Hester-Dendy samplers consist of a weight with 9 to 14 plates. The plates on each sampler are spaced at varying widths and have a total surface area of 0.16 m. The
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samplers will be placed in the ponds for approximately 6 to 8 weeks as the length of time for maximum colonization of the samplers is not known. During the time that the samplers are in the pond, they will be colonized by periphyton, and then macroinvertebrates will colonize the spaces between the plates and consume the periphyton. Upon collection of the samplers, the macroinvertebrates can be picked off the plates with pre-cleaned, plastic forceps and placed into sample containers.

The Hester-Dendy samplers will be attached to a cinder block to hold them under water and keep the samplers from getting buried in the sediments. Three samplers will be deployed around the pond. One will be near the influent end of the pond, the other near the discharge location. The last sampler will be deployed on either side of the pond half way between the other samplers. A rope from the cinder block to the shore will be used to assist in finding the samplers later.

Three replicate kick net samples will be collected from the perimeter of the sediment pond. The pond will be divided into three parts: up-gradient, middle and down-gradient. The middle will consist of both sides of the pond, below the influent end of the pond and above the discharge point. These locations will correspond with the location of the Hester-Dendy samplers.

Replicate samples will represent a composite of macroinvertebrates. The field samplers will make qualitative notes during the collection of replicate samples for the macroinvertebrates collected on the Hester-Dendy samplers and in the kick nets. These notes will include information about the functional feeding groups of the macroinvertebrates, as mentioned above in the discussion on sampling the wetlands. The field notes will be used to characterize the sample variability in the final report.

C. Sample Preparation and Preservation

Collection of a replicate sample in a wetland will continue until a minimum mass of 3 g wet weight macroinvertebrates has been collected. If the goal of collecting a mass of 3 g wet weight per replicate sample can not be achieved, then samples for a location may have to be composited prior to analysis.

Detritus and other plant material will be picked from the kick nets using pre-cleaned, plastic forceps. Deionized water will be used to rinse sediment from the net and the macroinvertebrates. The remaining macroinvertebrates will be transferred using pre-cleaned, plastic forceps onto a glass fiber filter (to remove excess water) and then into a labeled, pre-cleaned SPEX plastic container. The mass of the macroinvertebrate sample will be measured with a portable electronic balance (readability of 0.002g), and the weight will be recorded.

The macroinvertebrates removed from the Hester-Dendy samplers will be treated similar to the kick net samples. The organisms will be rinsed with deionized water and placed in a labeled, pre-cleaned SPEX plastic container. Sample mass will be measured with a portable electronic balance, and the weight will be recorded.
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All samples will be kept at 4°C during collection and shipping in order to preserve the sample until analysis. Standard chain of custody methods and labels will be used for all collected samples. Field data forms will also be used to document all pertinent sampling information.

D. Emergent, Flying Macroinvertebrates Sampling

In addition to aquatic macroinvertebrates, emergent, flying macroinvertebrates will be collected with blacklight traps. The light traps with photoelectric switches will be set up late in the day near each wetland and the sediment pond, and the location of the trap will be recorded with GPS. The sampling surfaces of each light trap will have been pre-cleaned with hydrochloric acid. After the lights have been in place over night, samples will be rinsed out of the light trap using deionized water, and the organisms will be collected on GF filter paper to drain off the water. A replicate sample will consist of at least 3 g wet weight. Multiple nights of collection may be necessary to collect the required mass of macroinvertebrates for each location. The samples will be collected in labeled, pre-cleaned SPEX plastic containers on ice while in the field and shipping, and then they will be frozen and archived until analyzed at a later date.

E. Quality Control of Field Sampling

To assess the quality of the field sampling technique, two types of field quality control samples will be collected:

- **Field Duplicates**: Field duplicates are used to assess the reproducibility of sample collection techniques. Typically, field duplicates would involve taking a separate sample at the same location. However, it is unlikely that enough organisms will be available at a location for two sampling efforts. Therefore, the field duplicate will be a split of a composite sample prepared after the determination of functional feeding groups. The split will ensure that a minimum of 3 g will be in each sample. Care will be taken to make field duplicates indistinguishable so that personnel performing analyses cannot determine which samples are duplicates. At a minimum, one field duplicate will be prepared for the Spring 2005 sampling event.

- **Field Blanks**: Field blanks are used to verify that the sample collection and handling process has not affected the quality of the samples. Field blanks are used to measure the cleanliness of sampling equipment. One field blank will be prepared in the field each sampling day by simulating the collection of samples for all types of media through decontaminated sampling equipment. Deionized water will be used for the field blank.

All field quality control samples are recorded as such in the field records. These quality control samples are analyzed by the laboratory to assess the quality of the sampling methodology. Field quality control samples will remain blind to the laboratory.
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F. Summary of Field Sampling Efforts

The field sampling efforts will collect the following samples, if at all possible:

- 3 aquatic macroinvertebrate samples collected with kick nets in wetland 1;
- 3 aquatic macroinvertebrate samples collected with kick nets in wetland 2;
- 3 aquatic macroinvertebrate samples collected with kick nets in wetland 3;
- 3 aquatic macroinvertebrate samples collected with kick nets in sediment pond;
- 3 aquatic macroinvertebrate samples collected from Hester-Dendy samplers in sediment pond;
- 1 field duplicate from composited aquatic macroinvertebrate sample;
- 3 emergent, flying macroinvertebrate samples collected with light traps in wetland 1;
- 3 emergent, flying macroinvertebrate samples collected with light traps in wetland 2;
- 3 emergent, flying macroinvertebrate samples collected with light traps in wetland 3;
- 1 field duplicate from composited emergent, flying macroinvertebrate sample; and
- 3-4 water samples collected as field blanks (one per day) and deionized water used for any re-wetting of macroinvertebrate samples.

V. Laboratory Test Analyses

The samples collected in the field will be analyzed for selenium.

A. Percent Moisture Determination

Samples collected in the field will have a wet weight, and the recommended ecological risk guidelines for macroinvertebrates are based on dry weight. To convert from wet weight to dry weight, the percent moisture in a sample must be determined. Percent moisture is determined as the percent ratio of wet to dry weight for the entire sample. Dry weights will be determined by placing the wet sample in a pre-tared, pre-cleaned sample container, lyophilizing (freeze drying) the entire sample, and then noting the change in weights. A sample of macroinvertebrates that weighs at least 3 g wet weight will be approximately 0.5 g dry weight.

B. Low-Level Trace Metals Analysis

The recommended analytical technique for analyzing selenium in macroinvertebrates is by atomic absorption, gaseous hydride procedure for low-level trace metals analysis (EPA Method 270.3; Standard Method 3114 B) (EPA 1982; APHA 1998). Sample preparation will include lyophilizing the samples and then homogenizing them using a ball-mill prior to digestion. An aliquot of approximately 0.5 g of each dried, homogeneous sample will be combined with nitric and hydrochloric acids (aqua regia) in a Teflon vessel and heated in an oven at 130°C (±10°C) for a minimum of eight hours. After heating and cooling, deionized water will be added to the acid-digested tissue to achieve analysis volume and the digestates will be submitted for analysis.

Digested samples will be analyzed for selenium using hydride generation flow injection atomic absorption spectroscopy (HGAA-FIAS). The base method for this procedure is EPA Method
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270.3 (EPA 1982). Samples that remain at 4°C or are frozen can be stored for up to a year prior to analysis. All results will be determined and reported in units of mg/kg on a dry-weight basis.

The detection limit for selenium in macroinvertebrate samples will be based on a methods detection limit (MDL) and reporting limit (RL) study to be performed by the analytical laboratory. MDLs for trace metals are typically determined annually by accredited laboratories in accordance with 40 CFR Part 136 Appendix B. MDLs for metals in tissue samples are generated by spiking tissue (e.g., cellulose or chicken breast) with low concentrations of each of the metals of interest, and processing them according to the laboratory methods. For trace metals, RL is calculated by multiplying the target analyte MDL by 3.18. The value 3.18 is based on the Student's-t value for 7 to 10 replicates, the number of replicates usually analyzed to generate the MDL. The laboratory chosen for the analysis of selenium in macroinvertebrate samples should be able to demonstrate a record of analyses for the following:

- MDL for tissues of 0.0112 mg/kg dry weight
- RL for tissues of 0.05 mg/kg dry weight

C. Laboratory Quality Control

Internal quality control is an important part of the measurement system to ensure that analytical results are reliable and that data integrity is maintained. Laboratory performance will be evaluated through analysis of laboratory quality control samples (in conjunction with field quality control samples, as appropriate).

Validation of the samples will evaluate the analytical performance of the laboratory by reviewing the results from analysis of the blank, matrix spike, duplicate, and quality control check samples. Evaluation will also be based upon instrumental calibration, instrument performance, adequacy of detection limits, obtained precision of replicate analyses, and comparison of the percentage of missing or undetected substances among replicate samples.

The following describes the batch preparative quality control samples that are required by the analytical method for low-level trace analysis of selenium.

- Method Blank (MB): A Method Blank consists of Type II ASTM water that is subjected to the sample preparation or extraction procedures and analyzed as a sample. It serves to measure contamination associated with laboratory storage, preparation, or instrumentation. One MB will be required for the 15 samples anticipated to be collected in Spring 2005 (typically, one sample is required for every 20 samples analyzed). If the analyte of interest is above the Reporting Limit, corrective action will be taken.

- Matrix Spike (MS): A Matrix Spike is an aliquot of sample to which known amounts of analyte have been added. It is subjected to the sample preparation or extraction procedures and analyzed as samples. The stock solutions used for spiking are purchased or prepared independently of calibration standards. One MS will be required for the 15 samples anticipated to be collected in Spring 2005 (typically, one sample is required for
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every 20 samples analyzed). The spike recovery measures the effects of interferences in the sample matrix and reflects the accuracy of the determination.

- **Matrix Spike Duplicate (MSD):** A Matrix Spike Duplicate is an additional aliquot of sample to which known amounts of analyte have been added and subjected to the same preparation and analytical scheme as the original sample. The Relative Percent Difference (RPD) between MS and MSD measures the precision of a given analysis. One MSD will be required for the 15 samples anticipated to be collected in Spring 2005 (typically, one sample is required for every 20 samples analyzed).

- **Laboratory Control Sample (LCS):** Laboratory Control Sample is created from a standard reference material which is a material similar in nature to the sample being processed [traceable to the National Institute of Standards and Technology (NIST) or other agencies, to the extent possible]. A known amount of analyte is added to an aliquot of Type II ASTM water. The LCS is subjected to the sample preparation or extraction procedure and analyzed as a sample. The stock solutions used for LCS recovery tests the function of analytical methods and instrumentation. One LCS will be required for the 15 samples anticipated to be collected in Spring 2005 (typically, one sample is required for every 20 samples analyzed).

If an adequate number of field duplicates can not be collected for the macroinvertebrate samples, laboratory splits may be substituted. In some cases, the mass may be too small to allow use of laboratory splits. Laboratory splits are used to assess the homogenization techniques. Samples are homogenized, and then divided into two equal parts for analysis. Care is taken to make both samples representative of materials present, including heterogeneities. If possible, at least one sample will be prepared and analyzed as a split.

Based on the number of samples anticipated to be collected in the field (see Section IV F above), at least one of each laboratory quality control sample will be analyzed for the aquatic macroinvertebrates to be collected in Spring 2005. Typically, the laboratory quality control samples are prepared for every 20 samples analyzed. The goal of for the minimum mass of 3 g wet weight is so that the analytical laboratory has enough mass to perform the MS or MDS to be performed on any sample. Laboratory quality control may have to be biased towards performing the MS or MDS on only those samples that have enough mass. Arrangements with the analytical laboratory about their ability to use samples with less than 3 g wet weight will be discussed prior to field sampling.

VI. Qualifications of Field Samplers

At a minimum, two people will be needed to collect the macroinvertebrate field samples in Spring 2005. The lead field sampler must have experience with collecting macroinvertebrate samples using kick nets, Hester-Dendy samplers and black light traps. In addition, the lead field sampler must have a background in entomology and be able to segregate macroinvertebrate samples into functional feeding groups. The second field sampler must have prior field experience with environmental sampling protocol, field documentation requirements, and be
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physically able to assist with deploying sampling equipment, sample collection and sorting techniques, and other related tasks as directed by the lead field sampler.

Deployment of the Hester-Dendy samplers will require two people. These people must have experience with the sediment retention pond, understanding the depth and flow of water through the pond as well as knowledge of past sampling activities at the pond. They must be able to deploy the samplers and ensure that the sampler is upright in the water column and not buried in the sediments.

VII. Schedule

There are two parts scheduled for the Spring 2005: 1) deployment of Hester-Dendy samplers in the sediment pond; and 2) collection of aquatic and flying macroinvertebrates from the wetlands and sediment pond. The optimum time for the collection of macroinvertebrates would be when the water temperature has warmed to optimum growing conditions and prior to emergence of aquatic insect larvae. Based on consultation with researchers that collect macroinvertebrates in the southeastern Utah, the optimum time for collection of macroinvertebrates is likely to be late May through early June (Axford 2004). Therefore, deployment of the Hester-Dendy samplers would be 6 to 8 weeks prior, or during the first two weeks of April.

Macroinvertebrate samples will be sent to the contract laboratory within 5 days after initiation of field collection activities. Laboratory results will be available approximately 45 days after the samples have been received.

VIII. Data Report

The data report will be submitted to DOE by September 30, 2005. The report will include the following information.

- A summary of the dates, times and locations of the field sampling activities
- Any communications with federal and state agencies, and professional biologists
- Any communications or direction from DOE
- A summary of the field activities, GPS data and any maps documenting required information
- A summary of the laboratory test analyses including results, methods, detection limits, and laboratory qualifications
- Education/qualifications of field samplers
- Comparison of results from macroinvertebrate samples to ecological risk guidelines and benchmarks
- Recommendation for follow-on activities
- References

The report will be reviewed by DOE and transmitted to EPA and UDEQ by November 1, 2005.
IX. Reference Information

The following reference information was used to develop this directive, or has been used by DOE and its contractor for other DOE sites. In many cases, it is not a complete citation because the reference was forwarded (in part) to DOE, and was not in a published format (e.g., faxed information, unpublished information).


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