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QUALITY ASSURANCE PROJECT PLAN FOR THE  
CALIFORNIA DEPARTMENT OF  
TOXIC SUBSTANCES CONTROL  
ARSENIC RELATIVE BIOAVAILABILITY STUDY  
August 3, 2009

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## INTRODUCTION:

This Quality Assurance Project Plan is for the Arsenic Relative Bioavailability Grant awarded to the California Department of Toxic Substances Control and funded by the United States Environmental Protection Agency as a Brownfield Training Research and Technical Assistance Grant, CERCLA section 104(k)(6).

Arsenic is the key chemical of concern at the majority of Brownfield projects at former gold mines in the California Mother Lode and Southern California desert areas. The Department of Toxic Substances Control (DTSC) intends to provide improved tools for assessment of health effects of arsenic at Mine-Scarred Lands (MSL). Specifically, DTSC intends to provide assessment tools to make full use of bioavailability of arsenic and resulting cost-effectiveness. Further, DTSC intends to provide tools for characterizing MSL sites so that bioavailability of arsenic can be employed in risk assessment and risk management.

Currently, toxicity criteria for estimating health effects of arsenic are based on humans exposed to arsenic dissolved in water. However, arsenic at MSL is bound to soil and rock. To properly assess arsenic risks at MSL, DTSC needs to describe its site-specific relative bioavailability (RBA), which is defined as the ratio of uptake of soil-bound arsenic to arsenic dissolved in water (a ratio of the arsenic that is absorbed in the body from the soil ingestion versus that portion that would be absorbed in the body from water ingestion). Current techniques for estimating bioavailability of arsenic are expensive and time-consuming. Animal studies (*in vivo* bioavailability) could be conducted at each site, but the costs would be prohibitive. Therefore DTSC intends to focus on how to predict bioavailability from inexpensive, routine measurements.

This Project will determine how arsenic is bound in soil, rock, and mine wastes at MSL sites and relate how the arsenic is bound in the solid matrix to *in vivo* bioavailability. A variety of chemical tests will be identified or developed that identify soil types which are connected to known *in vivo* bioavailability. These tests can then be used at MSL sites to predict arsenic RBA and accordingly calculate health risk and a health based site cleanup level for arsenic.

## SECTION 1.0, PROJECT OBJECTIVES AND ORGANIZATION

### 1.1. State the Project Objectives:

This Project will determine how arsenic is bound in soil (also rock, and mine wastes) at MSL sites and relate how the arsenic is bound in the solid matrix to *in vivo* bioavailability. A variety of chemical tests will be identified or developed that identify

soil types which are correlated to known *in vivo* bioavailability. These tests can then be used at MSL sites to predict arsenic RBA and accordingly calculate health risk and a health based site cleanup level for arsenic.

DTSC seeks to develop an assessment tool that would allow consultants and risk assessors to reliably predict the *in vivo* RBA of arsenic in soil samples from MSL sites in a scientifically sound, defensible, and cost-efficient manner. This assessment tool can then be used to characterize MSL sites. DTSC would produce an arsenic bioavailability guidance document that would assist in the proper characterization of MSL sites, however further studies beyond those described in this document will be necessary to achieve that end.

1.2. Table 1: Project Participants:

Project Participants	Primary Responsibility	Email	Mailing Address	Phone Number
Ann Carol, USEPA	EPA Project Officer	<a href="mailto:Carroll.Ann@epamail.epa.gov">Carroll.Ann@epamail.epa.gov</a>	USEPA Headquarters 1200 Pennsylvania Ave., 5105T Washington, DC 20460	202-566-2748
DTSC Investigators:				
Perry Myers	Principal Investigator	<a href="mailto:PMyers@dtsc.ca.gov">PMyers@dtsc.ca.gov</a>	DTSC 8800 Cal Center Dr. Sacramento, CA 95826	916-255-3708
Dr. John Christopher	Lead Technical Consult	<a href="mailto:JChristo@dtsc.ca.gov">JChristo@dtsc.ca.gov</a>	DTSC 8800 Cal Center Dr. Sacramento, CA 95826	916-255-6630
Dr. Valerie Mitchell	QAPP Preparation	<a href="mailto:VMitchel@dtsc.ca.gov">VMitchel@dtsc.ca.gov</a>	DTSC 8800 Cal Center Dr. Sacramento, CA 95826	916-255-6440
Brad Parsons	Project QC Officer	<a href="mailto:BParsons@dtsc.ca.gov">BParsons@dtsc.ca.gov</a>	DTSC 8800 Cal Center Dr. Sacramento, CA 95826	916-255-3661
Dr. John Quinn	Environmental Chemistry	<a href="mailto:JQuinn@dtsc.ca.gov">JQuinn@dtsc.ca.gov</a>	DTSC ESL 700 Heinz Avenue Berkeley, California 94710	510-540-2756
USGS Investigators:				
Dr. Charles Alpers	Geochemistry	<a href="mailto:cnalpers@usgs.gov">cnalpers@usgs.gov</a>	USGS, California Water Science Center Placer Hall, 6000 J Street Sacramento, CA 95819	916-278-3134
Dr. Dennis Eberl	Chemistry and Mineralogy	<a href="mailto:ddeberl@usgs.gov">ddeberl@usgs.gov</a>	USGS 3215 Marine St, Suite E-127 Boulder, CO 80303	303-541-3028

Dr. Andrea Foster	Geochemistry, X-ray Spectroscopy	<a href="mailto:afoster@usgs.gov">afoster@usgs.gov</a>	USGS. Mineral Resources Program 345 Middfield Rd, MS 901 Menlo Park, CA 94025	650-329-5437
Dr. Christopher Kim, Chapman University	Chemistry, Geochemistry, X-ray Spectroscopy	<a href="mailto:cskim@chapman.edu">cskim@chapman.edu</a>	Chapman University One University Rd Orange, CA 92866	714-628-7363
Dr. Nicholas Basta, Ohio State University		<a href="mailto:basta.4@osu.edu">basta.4@osu.edu</a>	The Ohio State University 2021 Coffey Rd Columbus, OH 43210-1085	614-292-6282
Dr. Stan Casteel, University of Missouri	<i>in vivo</i> Bioavailability of Arsenic in Juvenile Swine	<a href="mailto:CasteelS@missouri.edu">CasteelS@missouri.edu</a>	University of Missouri 201 Connaway Hall Columbia, Missouri 65211-5120	573-882-8120

## Project Organization

DTSC will provide overall project management for this project. Mr. Perry Myers will ensure that activities will be performed appropriately to meet project requirements. The investigators on the project will coordinate all activities through Dr. John Christopher and the DTSC Project Manager. Field sample collection activities will be supervised by DTSC in consultation with Dr. Charlie Alpers of the United States Geological Survey (USGS). The Quality Assurance Project Plan will be prepared by Dr. Valerie Mitchell and the project quality control officer will be Mr. Brad Parsons. California Environmental Quality Act activities will be supervised by Ms. Maria Gillette in coordination with the Empire Mine State Historic Park (EMSHP). Ms. Sandra Karinen will be responsible for contract management and public outreach. John Quinn is responsible for analytical services at the DTSC laboratory in Berkeley. He ensures that the laboratory complies with the QA and QC procedures outlined in the laboratory's QA Plan and the approved U.S. EPA methods of analysis. He will serve as the lead laboratory contact with the project manager for any issues related to project samples submitted for inorganic analysis.

DTSC is proud that we are partnering with leading researchers in their respective fields. Table 1 summarizes the different procedures being performed by the following investigators:

**United States Geological Survey:** Charles Alpers, Ph.D., Sacramento, CA, Dennis Eberl, PhD, Boulder, CO, and Andrea Foster, Ph.D., Menlo Park, CA, The USGS has tremendous experience in arsenic related studies and has access to and expertise in performing high energy spectral analysis and mineralogy. The USGS will assist with determining specific sample locations and will collect field notes on the properties of the samples. The USGS will analyze samples using bulk X-ray fluorescence, bulk X-ray diffraction, and X-ray diffraction using higher intensity X-rays.

**Chapman University:** Christopher Kim, PhD, Orange, CA. Dr. Kim will provide expertise in analytical chemistry in support of mineralogy. His efforts will include size fractionation, surface area analysis, speciation, and extraction of collected samples. Dr. Kim's work at the Randsburg Complex mining area will further our work in multiple extraction techniques to predict RBA.

**Ohio State University:** Dr. Nicholas Basta, PhD, Columbus, OH. Dr. Basta will provide his expertise to implement improvements of the *in vitro* simulated gastrointestinal assay for determining bioaccessibility of arsenic, and routine assays of samples from mine sites in California. Samples will be homogenized and sieved at Ohio State University before shipment to the other Study participants. Dr. Basta will perform *In vitro* bioaccessibility testing on the samples and provide residua of these tests to the USGS for further analysis.

**University of Missouri:** Dr. Stan Casteel, PhD, DVM. Dr. Casteel will provide his expertise to *in vivo* assays for determining bioavailability of arsenic. DTSC will work with Dr. Casteel to perform *in vivo* analysis of immature swine.

## SECTION 2.0, EXPERIMENTAL APPROACH

### 2.1 Experimental Process

This research will determine how arsenic is bound in soil, rock, and mine wastes at MSL sites and how the arsenic bound in the solid matrix relates to *in vivo* bioavailability. A variety of chemical tests will be identified or developed that identify soil types that correlate to known *in vivo* bioavailability. These tests can then be used at MSL sites to predict arsenic RBA and accordingly calculate health risk and a health based site cleanup level for arsenic. (RBA is a ratio of arsenic that is absorbed in the body from the soil ingestion versus that portion that would be absorbed in the body from water ingestion).

DTSC recognizes that arsenic is a widespread problem and proposes to focus this research on the western slope of the central Sierra Nevada Mountains in California, also known as the Mother Load. Sampling will occur principally at the Empire Mine State Historic Park (EMSHP), with the option of expanding to other MSL sample locations based on preliminary findings. The same geochemistry that created gold-bearing ores at this site also resulted in naturally occurring arsenic associated with high concentrations of iron oxides. There are a couple of reasons why additional sampling locations may need to be identified: 1) if we are unable to get the necessary range of arsenic bioaccessibility as determined from the *in vitro* analysis (eg a range of at least an order of magnitude) or 2) to obtain samples with different minearologic regimens to examine and potentially improve our model. These decisions will be made by the investigative group as a whole.

The general approach to the research is described in the following steps and the flow chart below (Diagram 1: Experimental Outline):

1. We intend to employ physical and wet chemical measurements to characterize soils from MSL. The selected measurements are those that other scientists have shown to be related to adsorption and release of arsenic from soil.
2. Bioaccessibility will be measured *in vitro* in a simulated gastro-intestinal system and based on those results select soil samples will be tested *in vivo* in juvenile swine to determine the RBA.
3. We will then construct a statistical model that correlates the *in vitro* and *in vivo* results. The goal is that this model can then be used to estimate RBA using the less expensive and time consuming *in vitro* analysis.
4. Measurements on many different soil types will be organized into a database. This database will be continuously expanded throughout the project.

## 2.2 Sampling Identification and Collection

A Field Sampling Plan has been prepared (See Appendix B) with the intention to identify soil and rock locations at selected MSL sites (Sites) appropriate for use in the RBA research project.

The Workplan includes the following.

- A generalized methodology for establishing the appropriate type, quantity, and location of samples for screening of arsenic related to historical mining activities at MSL Sites.
- A general description of the sampling techniques, for soil, including X-Ray Florescence (XRF) measurements, analytical methods, and quantity of samples to be taken.

### Targeted Locations

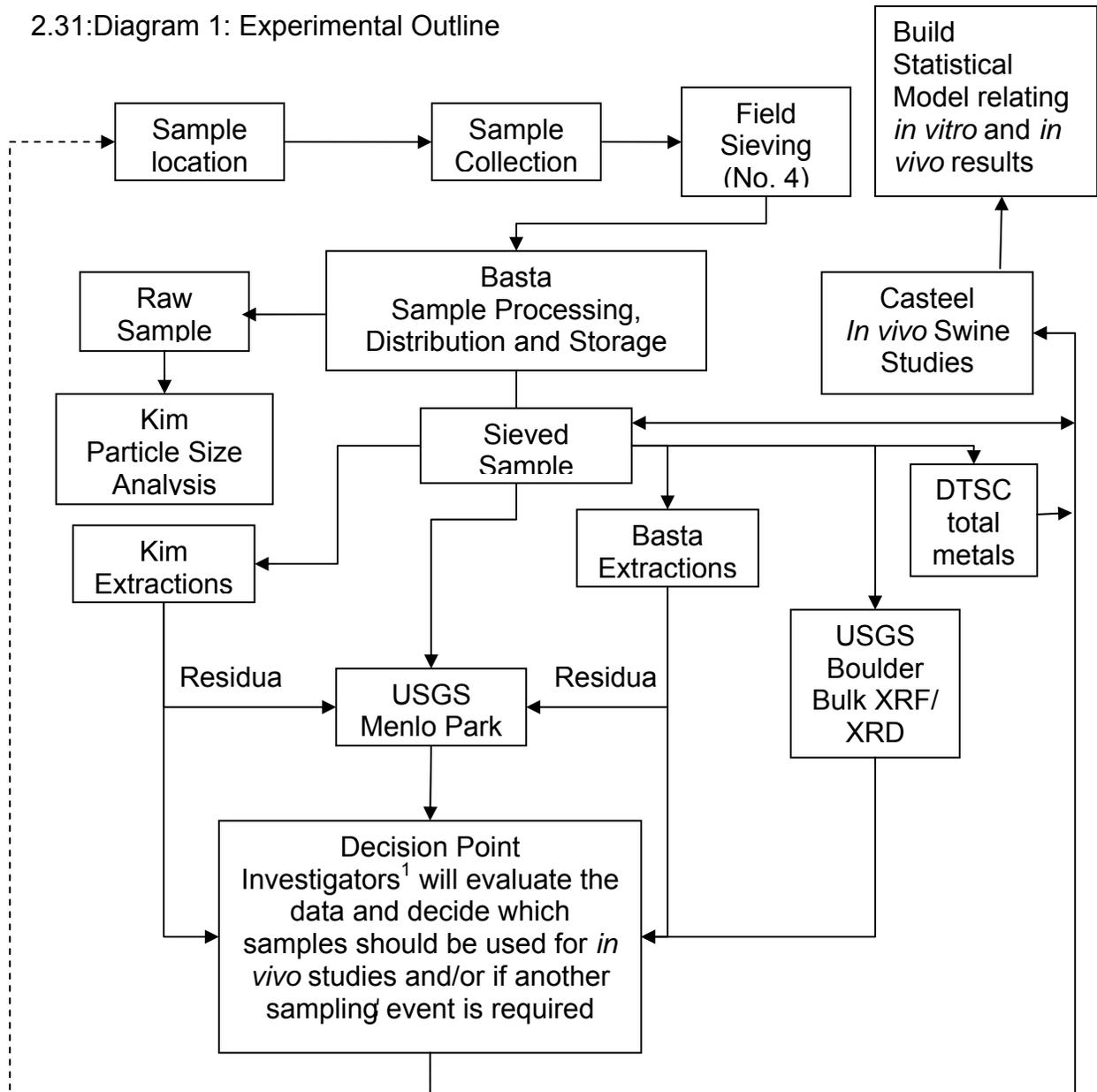
A reconnaissance sampling event was performed in April, 2009 with the following objectives:

1. Further explore overburden pile locations to identify areas that will fill in a range of arsenic concentrations and environments across the Park.
2. Locate, sample and analyze sample areas of native soil adjacent to mine shafts. (See Table 2 and Figure 3 of the Field Sampling Plan, Appendix B)
3. Locate, sample and analyze former mill and tailings areas including Sand Dam Area. (Table 2, Appendix B)
4. Observe field conditions and accessibility.
5. Create map of results including photographs.
6. Locate prospective sampling locations for next round of sampling.
7. Look for chemical trends within data (Fe/As and other ratios), (Table 2, Appendix B).
8. Present findings and determinations to work group.

Based on these reconnaissance samples, 24 locations have been identified as potential sources of material for the study. These locations are subject to change based on a number of factors including, but not limited to, restrictions by the EMSHP, inconsistencies with arsenic concentrations as detected by field XRF and/or the identification of more suitable sampling locations. Final sampling decisions will be made by DTSC consultation with Dr. Charlie Alpers of the USGS and the Department of Parks and Recreation (owner of the EMSHP).

## 2.3 Planned Approach for Project Objectives

2.31: Diagram 1: Experimental Outline



<sup>1</sup> The entire investigative group will meet via web-based teleconference to present data and discuss which samples will be moved through to the *in vivo* studies. This is a collaborative study and all investigators will be given equal weight in the decision making process.

### 2.3.2: Decision Point Analysis

We are trying to describe the cause and effect relationship between arsenic in soil and bioavailability of that same arsenic. Our predictors are (1) bulk arsenic content of soil; (2) mineralogical association of arsenic in soil; (3) mineralogical association of arsenic with species of iron oxide; (4) extractibility of arsenic in inorganic wet chemical systems; (5) extractibility of arsenic in a simulated intestinal environment; (6) arsenic content in different particle size fractions; (7) narrative information on bulk and micro-X-ray spectroscopy and X-ray diffraction. We expect to use multiple regression to identify which of these continuous variables significantly reduces the residual error of the regression ( $\alpha=0.05$ ). We will attempt to transform the narrative data to categories and use categorical regression to identify correlations arsenic content, bioavailability of arsenic, X-ray spectroscopy, and X-ray diffraction. After identifying statistical associations we will examine all our data using the Hill criteria to identify which factors are causally related to bioavailability of arsenic in this mineralogical system. Eventually, we hope to repeat these measurements and statistical procedures in different mineralogical regimes to test our predictive model.

### 2.3.3: Precision, Accuracy, Representativeness, Comparability, and Completeness:

Accuracy and precision of all arsenic measurements must remain below 5% in order to assure swine dosage precision of  $\pm 5\%$  per SOP #13. Evaluation will be based upon matrix spike (% recovery) and duplicate results (relative percent difference). Laboratory duplicates will be performed at a rate not less than 5% of analyses, and matrix spikes will occur at a minimum of one per sample batch not to exceed 20 samples per batch.

Representativeness of the samples will be achieved by authoritative selection and gathering of the samples, and a thorough homogenization of each sample lot. Each sample lot will be homogenized until a uniform variance is achieved amongst the subsamples. The lots representative of mining waste will be taken from the Empire Mine State Historic Park in Nevada County, California and are considered similar to gold mining wastes in this region of the Sierra Nevada Mountains. Appropriate quality assurance/quality control (QA/QC) samples will be collected, see Section 5.0, Quality Control/Quality Assurance, FSP, Appendix B, for details.

Comparability of data will be obtained by specifying standard units for physical measurements and standard procedures for sample collection, processing and analysis. Differences in procedures (e.g., drying temperatures) or laboratory biases (e.g., sieve efficiency) will be considered in evaluating the data generated.

Completeness (per cent of valid data) is not a quality objective for this project. Samples that will be collected and analyzed (up to a maximum of 60) as necessary to provide sufficient data for evaluation and to maximize use of available Synchrotron beam time. Sampling and analysis will continue until the critical numbers of sample analyses have been completed. Completeness is expected to exceed 80%.

The MDL's required for the various test methods are contained in the SOPs.

## SECTION 3.0, SAMPLING AND MEASUREMENT APPROACH AND PROCEDURES

### 3.1. Sampling Strategy.

The general sampling strategy is described in Section 2.1 and 2.2 of this document and detailed in Section 3 of the Field Sampling Plan, Appendix B.

### 3.2. Summary of the Sampling and Analytical Procedures

DTSC will ship the field-sieved samples to Dr. Basta at Ohio State University for further processing. Dr. Basta will send ~3 kg of each field-sieved sample to Dr. Kim for studies on particle size distribution, and sieve the remaining samples further to material  $\leq 250 \mu\text{m}$ . The investigative team assumes that the raw samples of soil will yield no less than 10-20% of fines by weight.

Dr. Basta will then distribute the  $\leq 250 \mu\text{m}$  material as shown below (all amounts refer to dry weight):

DTSC:	100 gm (22 metals including arsenic and iron)
Dr. Eberl:	100 gm (Bulk XRF/Bulk XRD)
Dr. Foster:	100 gm (Bulk XRD, X-ray spectroscopic studies)
Dr. Kim:	1,600 gm (Chemical Extractions)
Dr. Casteel:	1,000 gm (Feeding trials in swine)
Dr. Basta:	300 gm ( <i>in vitro</i> intestinal extraction/wet chemical extraction)

Dr. Basta will store all remaining processed and unprocessed material at Ohio State University.

Alternatively, if Dr. Basta and Ohio State are unable to perform this task, it will be performed by Dr. Christopher Kim at Chapman University.

The following diagrams represent the work being done by the various investigators (the SOP's can be found in Appendix A. When these data are collated for interpretation,

investigators will share their interpretations via web-based teleconferences. The data will then be compiled into the database maintained by DTSC personnel.

Diagram 3.2.1

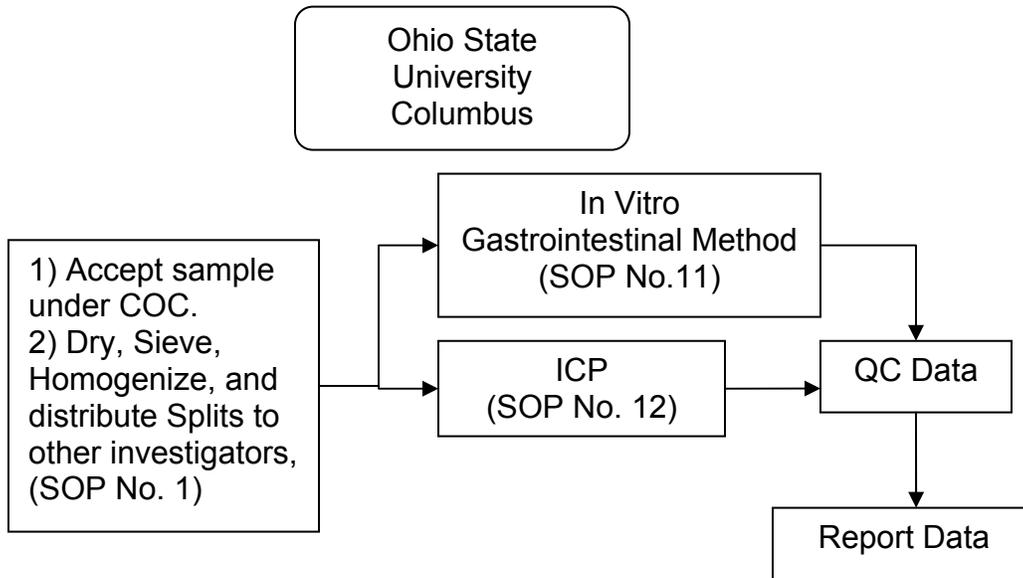
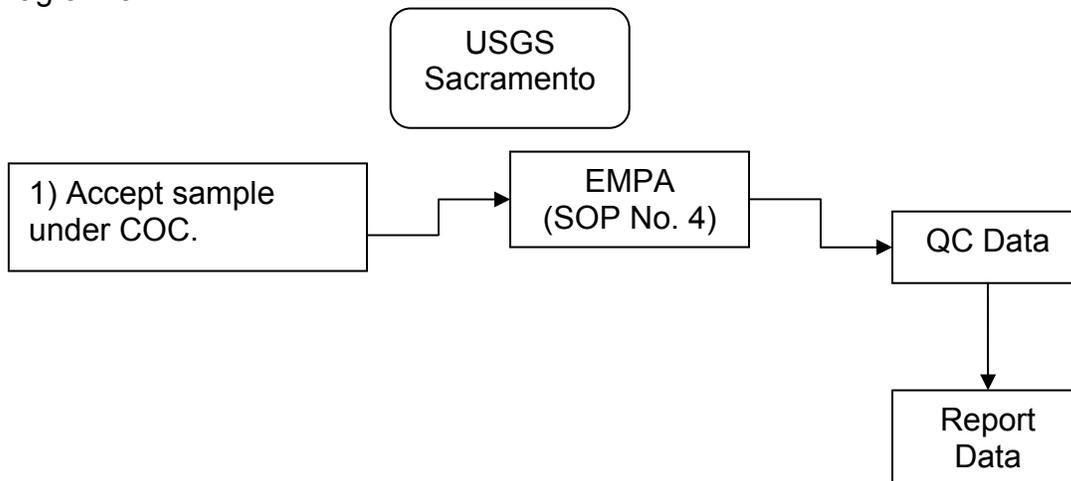


Diagram 3.2.2



Dr. Charlie Alpers with USGS, Sacramento will also be instrumental in the sampling collection events and will provide detailed field logs.

Diagram 3.2.3

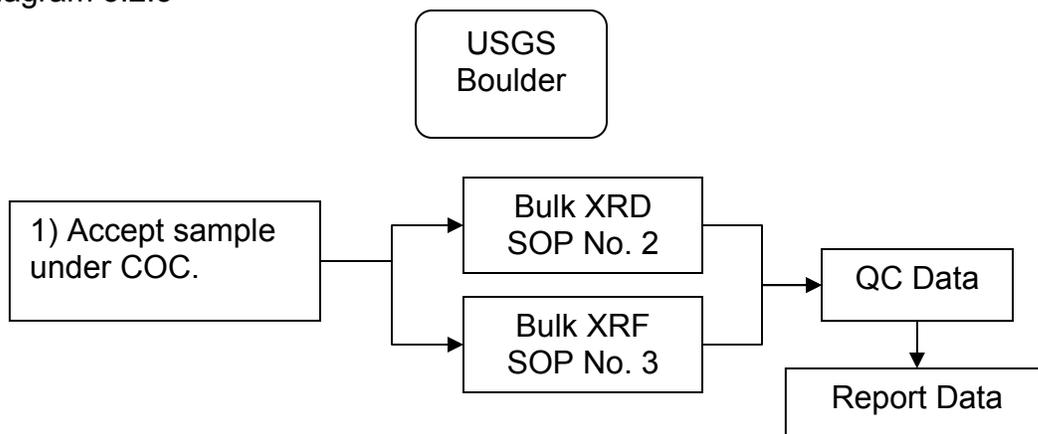
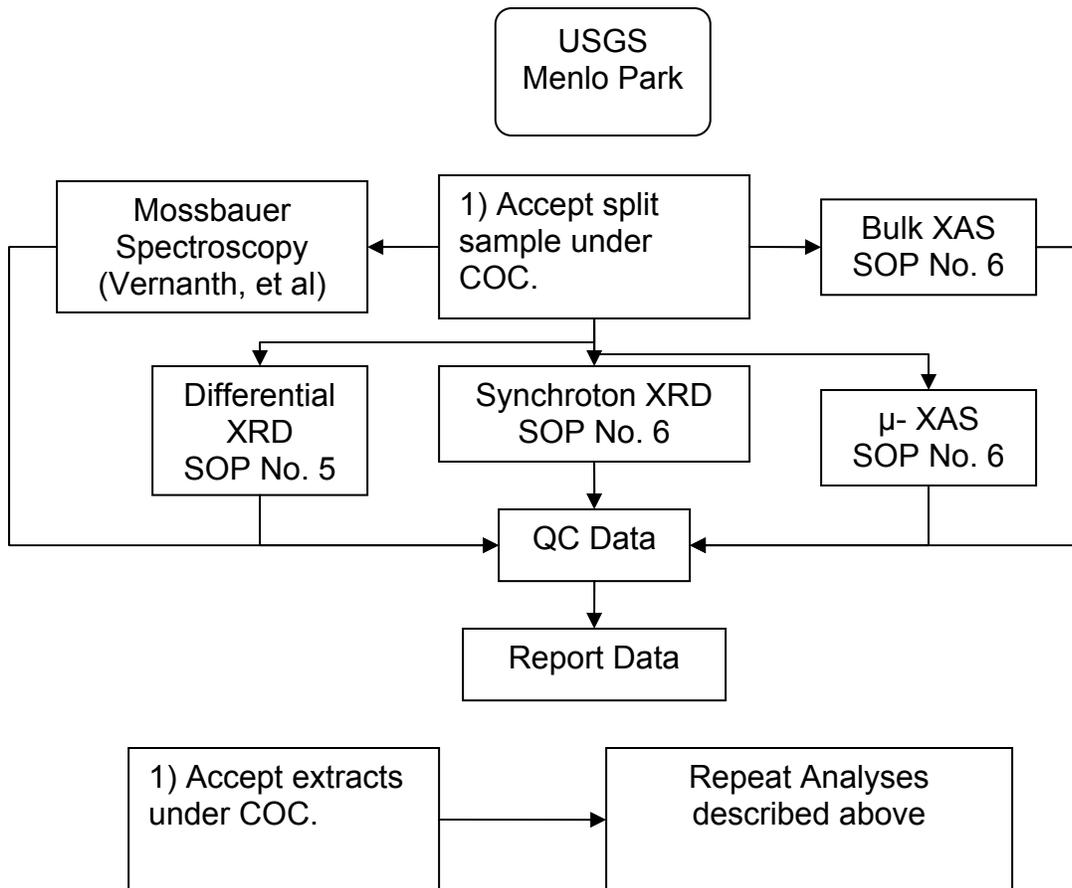


Diagram 3.2.4



The techniques employed by Dr. Andrea Foster at USGS, Menlo Park are quite complex and require a number of decision points throughout the analysis. A flowchart detailing her procedures for quantifying arsenic species and determining the mineralogy of iron (hydr)oxides in samples is depicted in Figure 1a and 1b below. Dr. Foster will be responsible for making the decisions indicated in the figures. Input will be provided by the rest of the investigative team as appropriate.

Figure 3.2.1a: Flowchart for quantifying arsenic species and determining mineralogy of Fe (hydr)oxides in samples from Mine-Scarred Lands

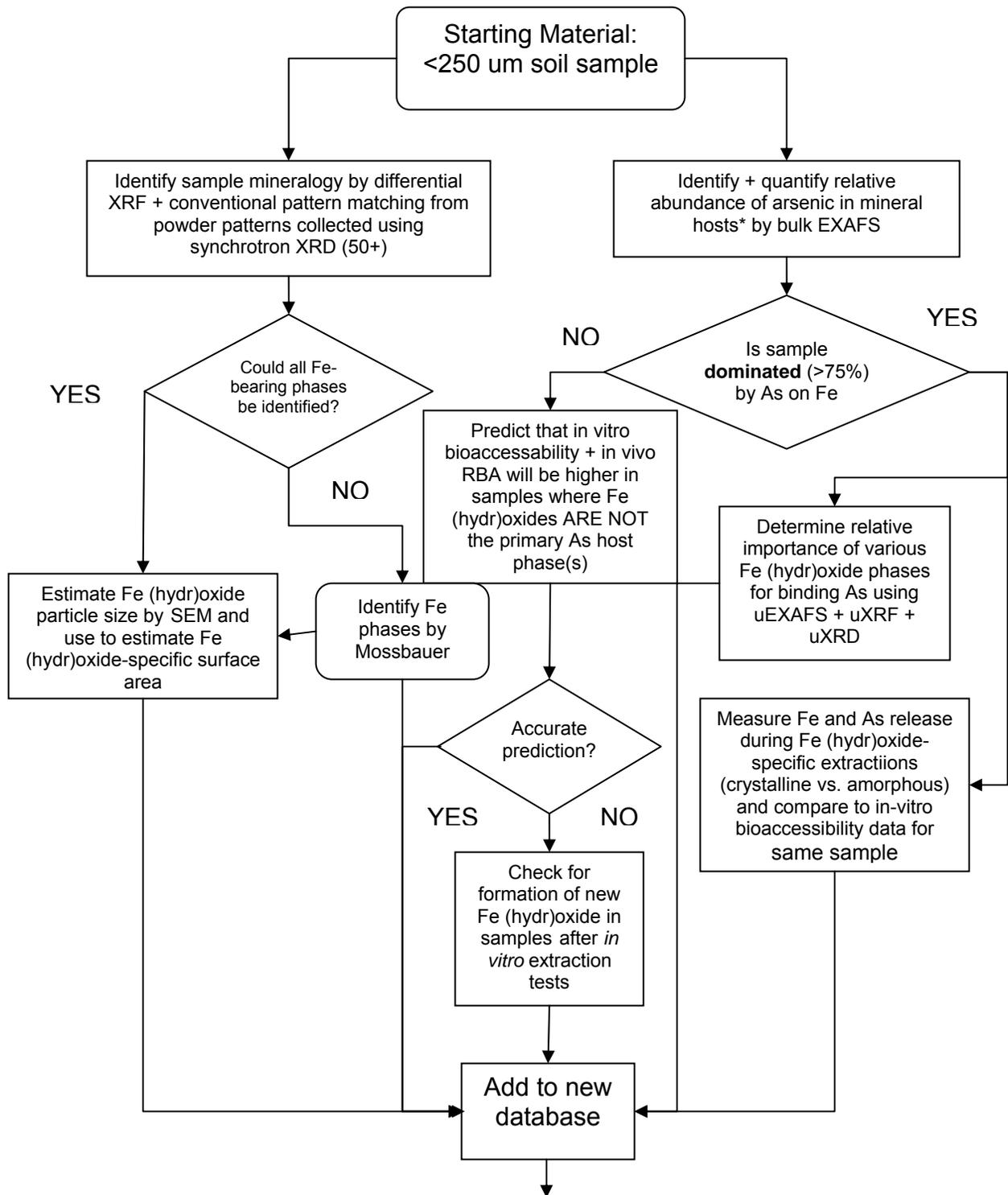


Figure 3.2.1b

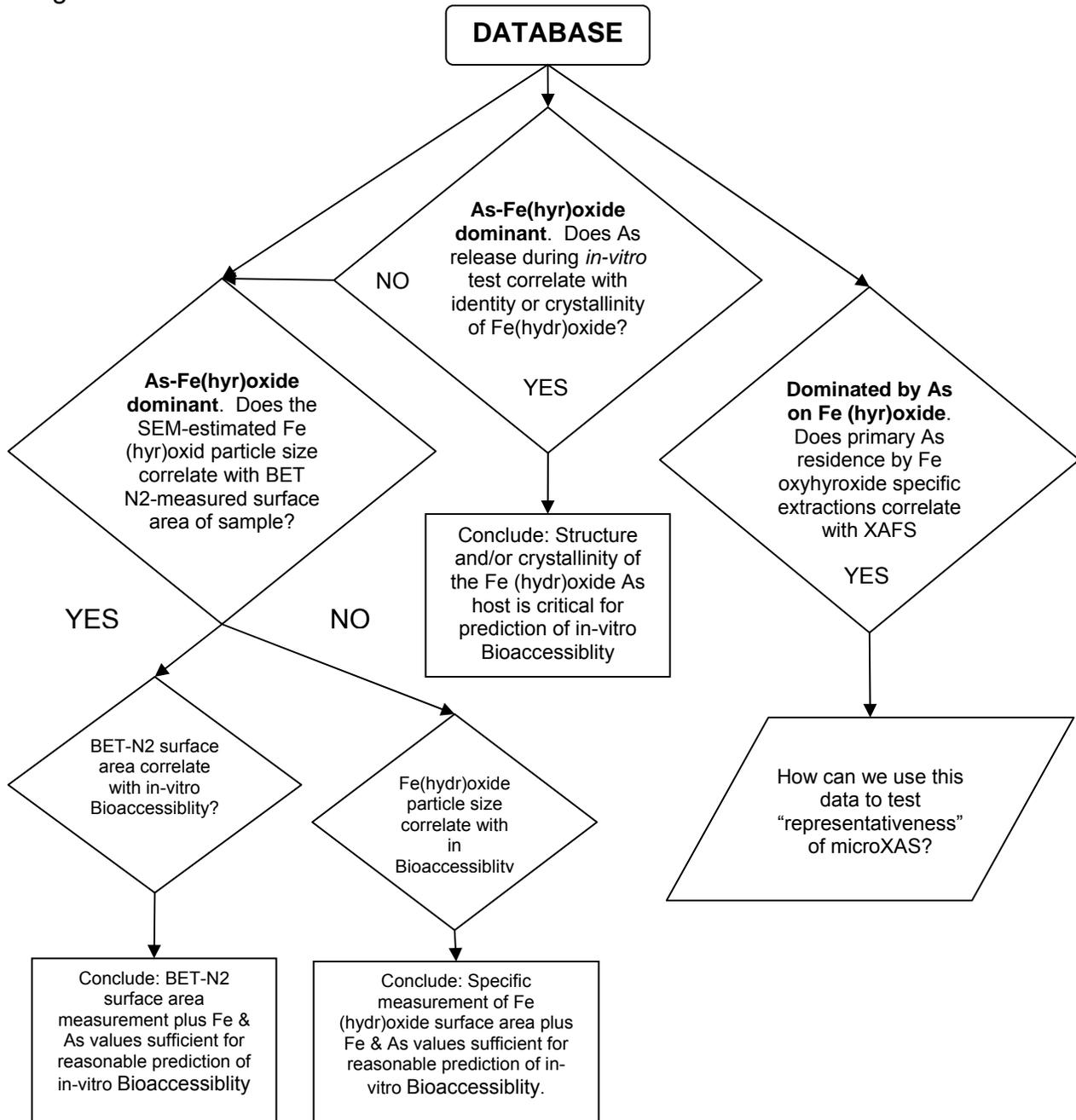


Diagram 3.2.5

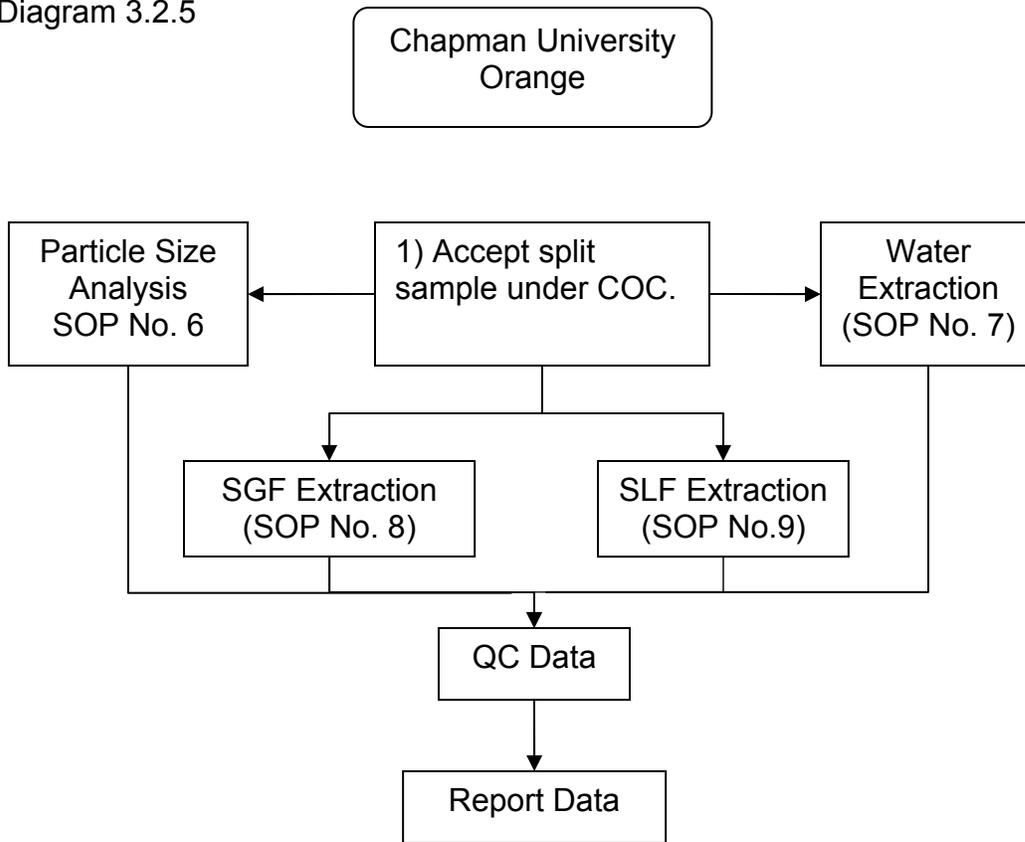
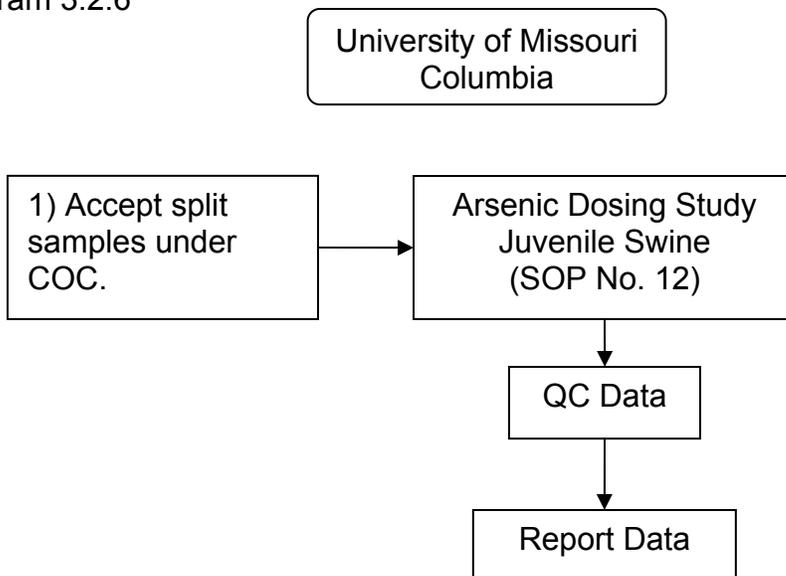


Diagram 3.2.6



## Section 4.0, QA/QC CHECKS

The quality assurance/ quality control aspects for each technique are covered in the individual SOPs that can be found in Appendix A.

Table 2: Summary Analyses and Associated Investigators

<b>Analysis Techniques</b>	<b>Associated Investigator</b>	<b>Associated SOP*</b>
<b>Bulk Soil Processing for Laboratory Studies</b>	Basta	SOP No. 1
<b>Bulk X-Ray Diffraction (XRD)</b>	Alpers/Eberl	SOP No. 2
<b>Bulk X-Ray Fluorescence (XRF)</b>	Alpers/Eberl	SOP No. 3
<b>Electron Microprobe Analysis (EPMA)</b>	Alpers	SOP No. 4
<b>Differential XRD</b>	Eberl/Foster	SOP No. 5
<b>Mössbauer Spectroscopy</b>	Foster/ Kim	Veranth, et al Reference
<b>Synchrotron-Based X-Ray Diffraction</b>	Foster	SOP No. 6
<b>Bulk X-Ray Absorption (XAS)</b>	Foster	
<b>μ-X-Ray Absorption Spectroscopy (μ-XAS) / μ-X-Ray Fluorescence Spectroscopy (μ-XRF) / μ-X-Ray Diffraction (μ-XRD):</b>	Foster	
<b>Particle Size Analysis</b>	Kim	SOP No. 7
<b>Water Extraction (ASTM, 2004)</b>	Kim	SOP No. 8
<b>Simulated Gastric Fluid (SGF) Extraction</b>	Kim	SOP No. 9
<b>Simulated Lung Fluid (SLF) Extraction</b>	Kim	SOP No. 10
<b><i>In Vitro</i> Gastrointestinal Method</b>	Basta	SOP No. 11
<b>Inductively Coupled Plasma (ICP) Spectrometry analysis</b>	Basta	SOP No. 12
<b>Arsenic Dosing Study, Juvenile Swine</b>	Casteel	SOP No. 13

## Section 5.0, DATA REPORTING

Each laboratory will perform an internal data check of results in accordance with the laboratories' standard quality assurance/quality control protocols. As applicable to each method, the following will be reviewed: Instrument performance, initial and continuing calibration verification, error determination (bias and precision), blanks results, compound identification, compound quantitation and reporting limits, performance evaluation sample results, and overall assessment of data. In addition each individual investigator will be responsible for reviewing any data that is manually entered into electronic format for accuracy. Data not meeting QC criteria will be appropriately flagged.

### **Laboratory Data Review**

In each laboratory analytical section, the analyst performing the tests shall review 100 percent of the definitive data. After the analyst's review has been completed, 100 percent of the data shall be reviewed independently by a senior analyst or by the supervisor of the respective analytical section using the same criteria.

The following elements for review/verification at each level must include but not be restricted to:

- Sample receipt procedures and conditions.
- Sample preparation.
- Appropriate SOPs and analytical methodologies.
- Accuracy and completeness of analytical results.
- Correct interpretation of all raw data, including all manual integrations.
- Appropriate application of QC samples and compliance with established control limits.
- Verification of data transfers.
- Documentation completeness (e.g., all anomalies in the preparation and analysis have been identified, appropriate corrective actions taken, and have been documented in the case narrative(s), associated data have been appropriately qualified, anomaly forms are complete).

### **Data Qualifiers**

Data qualifiers shall be applied by the laboratory for any data falling outside quality control criteria. The qualifiers will be defined in the laboratory report. The qualifiers will be compared to the project qualifiers by the QC Officer and reflagged as appropriate for the final project report. The flagging definitions for the project are contained in table 5.1 below.

Table 5.1 Laboratory Data Qualifiers

Qualifier	Description
Q	One or more quality control criteria (for example, LCS recovery, surrogate spike recovery) failed. Data must be carefully assessed by the project team with respect to the project-specific requirements and evaluated for usability. Subsequent assessment by the QC Officer or project team may result in rejection of data.
M	Matrix effect: The concentration is estimated due to a matrix effect.
J	Estimated: The analyte was positively identified, the quantitation is an estimation due to discrepancies in meeting certain analyte-specific quality control criteria.
F	Found: The analyte was positively identified but the associated concentration is estimation above the MDL and below the RL (or lowest calibration standard).
B	Blank contamination: The analyte was found in an associated blank above one half the RL, as well as in the sample.
U	Undetected: The analyte was analyzed for, but not detected.
UJ	The analyte was not detected; however, the result is estimated due to discrepancies in meeting certain analyte-specific quality control criteria.
R	The sample results are rejected due to serious deficiencies in the ability to analyze the sample and meet quality control criteria. Data is unusable for project purposes.

All data will be presented in a standardized format, provided electronically, and will include quality control results. When appropriate, a summary of the data should be provided in addition to the complete data set. A case narrative will be provided identifying any unexpected or conflicting results, unusable data, field or laboratory interferences, and any other matter affecting the use of the data. The project QC Officer, Mr. Brad Parsons, will review all of the data submitted from the various laboratories. The review will entail determining completeness, consistency of units, uniform identification of flagged data exceeding QC criteria, and summarizing any limitations on use of the data. Statistical calculations will be checked for accuracy.

Each laboratory will maintain records for a period of two years. The records will encompass sample data, sample management, test methods and QA/QC reports. These records allow for verification of the chain-of-custody, analytical methods with anomalies noted, sample preparation and analysis, instrument calibration, test specific criteria, detection limits, and various QC checks.

## SECTION 6.0, REFERENCES

Note: References for the various techniques are located at the end of each respective SOP, see Appendix A.