

Corona and Twin Peaks Mine Drainage Treatment Project

Department of Fish and Wildlife

Ecosystem Restoration Program Grant

ERP Grant # E1596004

Quality Assurance Project Plan (QAPP)

For

Mine Drainage, Surface Water, and Biota Sampling

with UC Davis Biosentinel Monitoring QAPP and SOPs

May 2016

Prepared by:

Beth Kelly, Burleson Consulting, Inc.

Peter Green, UC Davis

Tim Tsukamoto, TKT Consulting, LLC

Greg Reller, Burleson Consulting, Inc.

For:

Tuleyome, Inc.

QAPP Revision Number 1.0

Group A Project Management



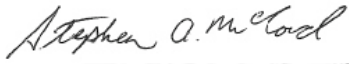




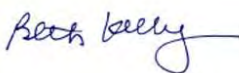
1.0 APPROVAL SIGNATURES

QAPP

Corona and Twin Peaks Mine Drainage Treatment Project

ERP Grant E1596004

May 2016

Title:	Name:	Signature:	Signature Date:
ERP Grant Manager	Erin Aquino-Carhart		<u>5/5/2016</u>
Tuleyome Project Lead	Sara Husby		<u>5/4/2016</u>
McCord Environmental President	Stephen McCord		<u>5/4/2016</u>
TKT Consulting, LLC Principal Chemist	Timothy Tsukamoto		<u>5/4/2016</u>
UC Davis Research Engineer	Peter Green		<u>5/4/2016</u>
Burleson Consulting, Inc. Senior Geologist	Greg Keller		<u>5/17/2016</u>
UC Davis Researcher	Darrell Slotton		_____
UC Davis Researcher	Vic Claassen		<u>5/5/2016</u>
Burleson Consulting, Inc. QA Officer	Beth Kelly		<u>5/4/2016</u>
Regional Water Quality Control Board SWAMP QA Review	Melissa Morris or OIMA Help Desk	(Review only)	_____

2.0 TABLE OF CONTENTS

Group A Project Management	i
1.0 APPROVAL SIGNATURES.....	i
2.0 TABLE OF CONTENTS.....	iii
3.0 DISTRIBUTION LIST	vii
4.0 PROJECT/TASK ORGANIZATION.....	1
4.1 Involved Parties and Roles.....	1
4.2 Quality Assurance Officer Role.....	2
4.3 Persons Responsible for QAPP Update and Maintenance.....	3
4.4 Organizational Chart and Responsibilities.....	3
5.0 PROBLEM DEFINITION/BACKGROUND.....	5
5.1 Problem Statement	5
5.2 Decisions or Outcomes.....	5
5.3 Water Quality or Regulatory Criteria.....	6
6.0 PROJECT/TASK DESCRIPTION	7
6.1 Work Statement and Produced Products.....	7
6.1.1 Preconstruction Studies.....	7
6.1.2 Treatment Facility Monitoring and Adaptive Management	8
6.2 Constituents Monitored and Measurement Techniques.....	9
6.3 Project Schedule.....	11
6.4 Geographical Setting.....	13
6.5 Constraints.....	14
7.0 QUALITY OBJECTIVES AND CRITERIA FOR MEASUREMENT DATA.....	15
7.1 Precision and Accuracy.....	15
7.2 Detection Limits.....	15
7.3 Completeness	15
7.4 Representativeness	15
7.5 Comparability.....	16
8.0 SPECIAL TRAINING NEEDS/CERTIFICATION.....	19
8.1 Specialized Training or Certifications.....	19
8.2 Training and Certification Documentation	19
8.3 Training Personnel	19
9.0 DOCUMENTS AND RECORDS.....	20

9.1	Field Documentation	20
9.2	Chain-of-Custody Records	22
9.3	Analytical Laboratory Records	22
9.4	Characterization and Monitoring Program Record Files	23
9.5	Field Change Request Forms	23
Group B Data Generation and Acquisition		24
10.0	SAMPLING PROCESS DESIGN	24
10.1	Preconstruction Studies	24
10.2	Treatment Facility Monitoring and Adaptive Management	24
11.0	SAMPLING METHOD	27
11.1	Field Sampling Techniques	27
11.1.1	Surface Water Field Measurements	27
11.1.2	Fish and Aquatic Insect Sampling	27
11.1.3	Mine Drainage and Surface Water Sampling	27
11.2	Decontamination	27
11.3	Sample Containerization, Preservation, and Holding Times	28
12.0	SAMPLE HANDLING AND CUSTODY	30
12.1	Sample Labeling and Handling	30
12.2	Chain-of-Custody Procedures	32
12.3	Laboratory Operations	32
12.3.1	Sample Receipt	32
12.3.2	Sample Storage and Security	33
12.3.3	Sample Tracking	33
12.3.4	Sample Custody Records	34
13.0	ANALYTICAL METHODS	35
14.0	QUALITY CONTROL	37
14.1	Field Quality Control Samples	37
14.1.1	Field Duplicate Samples	37
14.1.2	Field Method Blank Samples	37
14.1.3	Field Equipment Rinsate Blank Samples	37
14.2	Laboratory Quality Control Samples	37
14.2.1	Method Blanks	38
14.2.2	Matrix Spikes	38
14.2.3	Laboratory Control Samples (Verification Solutions)	38

14.2.4	Laboratory Duplicate Samples.....	38
15.0	INSTRUMENT/EQUIPMENT TESTING, INSPECTION, AND MAINTENANCE.....	41
15.1	Calibration of Sampling Equipment.....	41
15.2	Calibration of Laboratory Equipment.....	41
16.0	INSTRUMENT/EQUIPMENT CALIBRATION AND FREQUENCY.....	42
16.1	Calibration Methods.....	42
16.2	Calibration Apparatus.....	42
16.3	Calibration Standards.....	42
16.3	Calibration Frequency.....	43
17.0	INSPECTION/ACCEPTANCE OF SUPPLIES AND CONSUMABLES.....	50
18.0	NON-DIRECT MEASUREMENTS (EXISTING DATA).....	51
19.0	DATA MANAGEMENT.....	52
19.1	Field Data Verification and Reporting.....	52
19.2	Laboratory Data Verification/Validation.....	52
19.3	Data Storage and Retrieval.....	53
	Group C Assessment and Oversight	54
20.0	ASSESSMENTS AND RESPONSE ACTIONS.....	54
20.1	Field and Laboratory Assessments.....	54
20.2	Independent Technical Review.....	54
20.3	Documentation of Assessments.....	55
20.4	Corrective and Preventive Action.....	55
21.0	REPORTS TO MANAGEMENT.....	57
	Group D Data Validation and Usability	58
22.0	DATA REVIEW, VERIFICATION, AND VALIDATION REQUIREMENTS.....	58
22.1	Data Review and Verification Requirements.....	58
22.2	Validation Methods.....	58
23.0	VERIFICATION AND VALIDATION METHODS.....	60
23.1	Data Validation Reports.....	60
24.0	RECONCILIATION WITH USER REQUIREMENTS.....	61
	ACRONYMS AND ABBREVIATIONS.....	62
	REFERENCES.....	65

List of Tables

Table 4-1	QAPP Personnel Responsibilities
Table 6-1	Data Quality Objectives of Corona and Twin Peaks Mercury Mines Project, Napa County, California
Table 6-2	Project Schedule and Deliverables
Table 7-1	Aqueous Metal Detection Limits and Screening Criteria
Table 7-2	Field Analytical Methods and Detection Limits
Table 7-3	Laboratory Measurement Quality Objectives for Analytical Parameters for Water
Table 10-1	Field Sampling Locations and Analytical Methods
Table 11-1	Analytical Laboratory Sample Analysis, Volumes, Holding Times, and Preservation Requirements
Table 12-1	Surface Water Sample Locations and Identifications
Table 13-1	Summary of Analytical Parameters and Methods
Table 14-1	Summary of Laboratory Quality Control Samples
Table 16-1	Summary of Laboratory and Field Calibration Requirements for Major Parameters
Table 21-1	QA Management Reports
Table 22-1	Definitions of Data Flags and Qualifiers for Inorganic Data

List of Figures

Figure 4-1	Grant Project Organization and Personnel Responsibilities
------------	---

Appendices

Appendix A	UC Davis Mercury Bioaccumulation Monitoring for the California Bay-Delta Authority Quality Assurance Project Plan and Standard Operating Procedures
------------	---

3.0 DISTRIBUTION LIST

<u>Title:</u>	<u>Name (Affiliation):</u>	<u>E-mail</u>	<u>Telephone Number</u>
ERP Grant Manager	Erin Aquino-Carhart (DFW)	Erin.Aquino-Carhart@wildlife.ca.gov	916-445-1287
Project Lead	Sara Husby (Tuleyome, Inc.)	sdhusby@tuleyome.org	530-350-2599
Project Manager	Stephen McCord (McCord Environmental, Inc.)	sam@mccenv.com	530-220-3165
Analytical Chemist	Peter Green (UC Davis)	pggreen@ucdavis.edu	530-752-8581
Biomonitoring Specialist	Darrell Slotton (UC Davis)	dgslotton@ucdavis.edu	530-756-1001
Revegetation Specialist	Vic Claassen (UC Davis)	vpclaassen@gmail.com	530- 902-4622
Chemical Treatment Specialist	Tim Tsukamoto (TKT Consulting, LLC)	tkttim@gmail.com	775-846-9659
Mine Reclamation Specialist	Greg Reller (Burleson Consulting, Inc.)	gr@burlesonconsulting.com	916-984-4651
QC Officer	Beth Kelly (Burleson Consulting, Inc.)	bk@burlesonconsulting.com	916-984-4651
Laboratory	Todd Albertson (Caltest Analytical Laboratory)	todd@caltestlabs.com	707-258-4000
Site Manager	Justin Smith (Justin Smith Construction)	jsoathill@gmail.com	707-486-6367

4.0 PROJECT/TASK ORGANIZATION

This project supports the scope of work for Grant Number E1596004 (Grant), an agreement with the State of California Department of Fish and Wildlife (DFW), under their Ecosystem Restoration Program (ERP) (DFW, 2011). This project was originally awarded in 2012 under Grant E1183005; however, the project was not completed because the drainage treatment method was different than the original scope. Therefore, this QAPP has been prepared to support the project awarded under Grant E1596004. This project includes preconstruction studies and treatment facility monitoring at the Corona and Twin Peaks Mercury Mines in Napa County. This document is the Quality Assurance Project Plan (QAPP) for the project, prepared utilizing the Surface Water Ambient Monitoring Program (SWAMP) compatible template (State Water Resources Control Board [SWRBC], 2008).

The Corona and Twin Peaks Mercury Mines (sites) are located on private property in Napa County, California and are about 7 miles southeast of Middletown, California, and about 10 miles north of St. Helena, California. The sites are at an elevation of about 1,900 feet above mean sea level (amsl) and the topography is relatively steep and forested. The mines are located about 0.75 mile apart along the Oat Hill Road. The sites are on contiguous parcels consisting of about 32 acres. The project will improve the effectiveness of existing mine drainage treatment systems at the Boiler House and Twin Peaks adits, minimize leaching and mobilization of nickel and acid drainage from the mined ore body through the Corona Drain Tunnel, and address site physical and chemical hazards so that the sites are safe for recreational use and support a healthy aquatic ecosystem. The property is an important component in the Napa County Open Space and Trails Plans, and would connect two pieces of the Palisades public lands and eventually connect a trail to Lake County (DFW, 2011).

4.1 Involved Parties and Roles

Erin Aquino-Carhart, DFW, is the Grant Manager. Sara Husby of Tuleyome, Inc. (Tuleyome) is the project lead and Stephen McCord of McCord Environmental, Inc. (MEI) is the project manager. Peter Green of University of California Davis (UC Davis), in coordination with Tim Tsukamoto of TKT Consulting, LLC (TKT), will organize the sample collection, field sampling, and in-house analysis of samples for surface water characterization. Darrell Slotton of UC Davis, will collect and analyze aquatic biota samples.

Peter Green, Tim Tsukamoto, and Greg Reller are responsible for organizing field staff, conducting the tracer study, conducting the pilot-scale operation, scheduling of sampling days, managing the UC Davis laboratory, and interacting with contract laboratories for surface water sampling. Darrell Slotton is responsible for coordination with UC Davis for analyzing fish and aquatic insects. Vic Claassen is responsible for coordination with the team for vegetation and soil nutrient data from previous tasks under the original Grant for this project.

UC Davis will be the contract laboratory for analyses of surface water, soil, waste, fish, and aquatic insect samples. Caltest Analytical Laboratory, located at 1885 North Kelly Road, Napa, California, will analyze samples for low level methyl mercury. A&L Western Agricultural

Laboratories, Inc. located at 1311 Woodland Avenue, Suite #1, Modesto, California will analyze samples for soil nutrients, as needed. The laboratories will analyze submitted samples in accordance with the methods and quality assurance requirements found in this QAPP and the UC Davis *QAPP for Mercury Bioaccumulation Monitoring for the California Bay-Delta Authority (CBDA)* (see Appendix A) (Slotton, D.G., et al., 2008). Table 4-1 provides the list of personnel and their responsibilities.

Table 4-1 QAPP Personnel Responsibilities

NAME	ORGANIZATIONAL AFFILIATION	TITLE/ ROLE	CONTACT INFORMATION
Sara Husby	Tuleyome, Inc.	Project Lead	530-350-2599 sdjusby@tuleyome.org
Stephen McCord	McCord Environmental, Inc. (MEI)	Project Manager	530-220-3165 sam@mccenv.com
Peter Green	UC Davis	Researcher/ Analytical Chemist	530-219-9023 pggreen@ucdavis.edu
Tim Tsukamoto	TKT Consulting, LLC	Chemist/Chemical Treatment Specialist	775-846-9659 ttkim@gmail.com
Darrell Slotton	UC Davis	Researcher/ Biomonitoring Specialist	530-756-1001 dgslotton@ucdavis.edu
Vic Claassen	UC Davis	Researcher/ Revegetation Specialist	530-902-4622 vpclaassen@gmail.com
Greg Reller	Burleson Consulting, Inc.	Geologist/ Mine Reclamation Specialist	916-984-4651 ext 111 gr@burlesonconsulting.com
Beth Kelly	Burleson Consulting, Inc.	QA Officer/ Chemist/Data Review	916-984-4651 ext 114 bk@burlesonconsulting.com

4.2 Quality Assurance Officer Role

Beth Kelly is the Quality Assurance (QA) Officer. She is a chemist with over 25 years of environmental and chemical analytical experience, including data validation. Her role is to oversee the quality assurance/quality control (QA/QC) procedures found in this QAPP as part of the sampling, field analysis, and in-house analysis procedures.

The QA Officer will also review and assess all procedures during the life of the contract against QAPP requirements. She may stop all actions if there are significant deviations from required practices or if there is evidence of a systematic failure and will report all findings to Stephen McCord, Project Manager, including all requests for corrective action.

4.3 Persons Responsible for QAPP Update and Maintenance

Changes and updates to this QAPP may be made after a review of the evidence for change by the Project Manager and the QA Officer, and with the concurrence of the DFW. The QA Officer is responsible for review of changes, submitting drafts for review, preparing a final copy, and submitting the final QAPP for signature.

The Mine Reclamation Specialist is responsible for conducting the pilot-scale operations and assisting with treatment design. He is responsible for updating the QAPP for any changes to these tasks or monitoring.

The Chemical Treatment Specialist is responsible for tracer study and pilot scale operations. The Analytical Chemist is responsible for sampling surface water and providing the analytical data in the QAPP format and updates to the sampling locations and analytes.

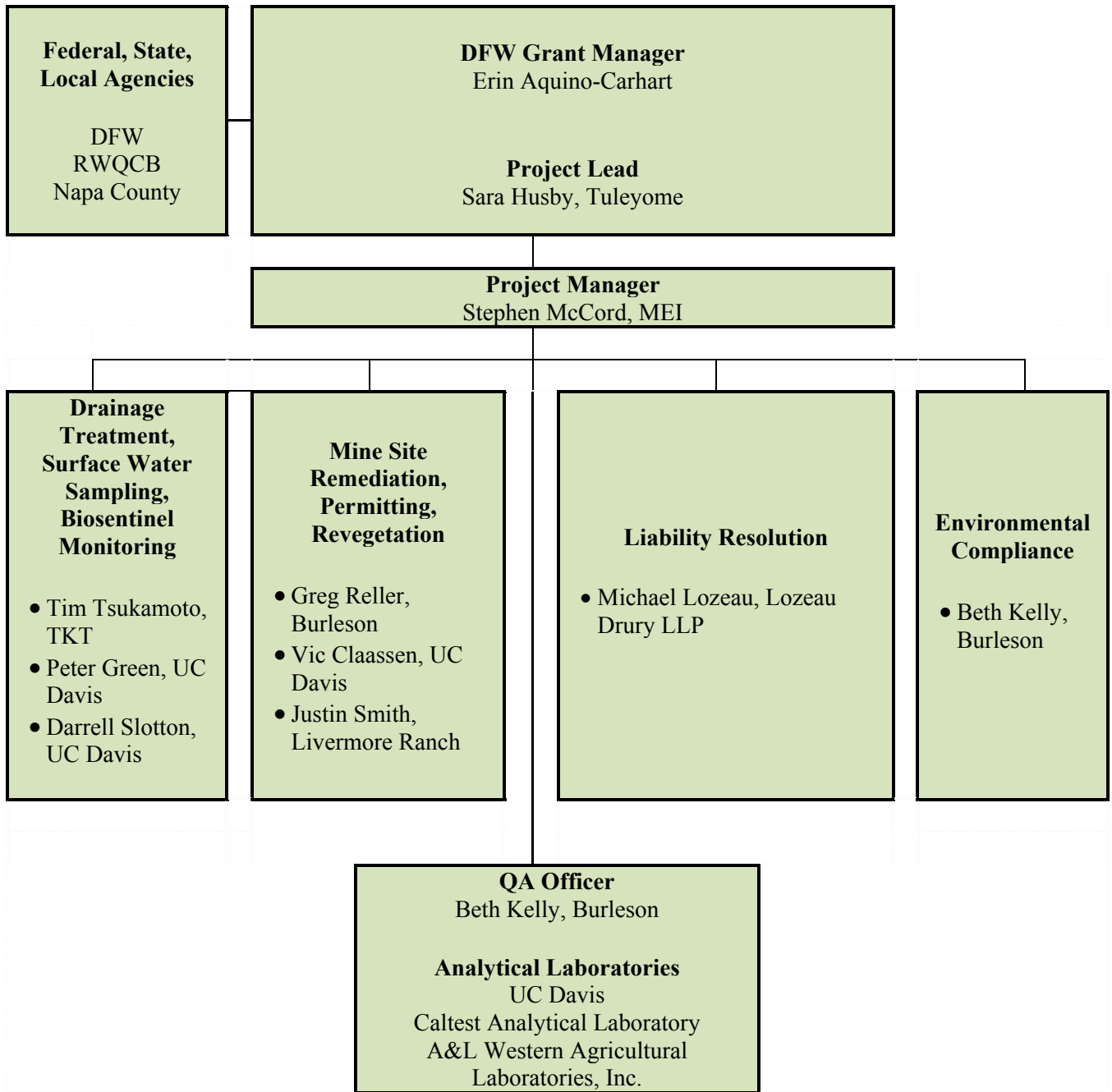
The Biomonitoring Specialist is responsible for revisions to the QAPP for fish and aquatic insect sampling and analyses.

The Revegetation Specialist is responsible for revisions to the QAPP for soil nutrient analyses, if needed. However, soil sampling is currently not included in this QAPP.

4.4 Organizational Chart and Responsibilities

Please see Table 4-1 for QAPP responsibilities. The Grant project organizational chart and personnel responsibilities are shown below in Figure 4-1.

Figure 4-1 Grant Project Organization and Personnel Responsibilities



5.0 PROBLEM DEFINITION/BACKGROUND

The DFW Grant Ecosystem Restoration Program project goals are to: (1) render the project site safe for public use; and (2) support healthy aquatic ecosystems downstream in James Creek, Pope Creek, Lake Berryessa, lower Putah Creek, lower Yolo Bypass, and the Delta. The project objectives are: (1) improve the effectiveness of existing mine drainage treatment systems for the Boiler House and Twin Peaks adits; (2) minimize leaching and mobilization of nickel and acid drainage from the mined ore body through the Corona Drain Tunnel; and (3) address physical and chemical hazards on the site. This project is a continuation under the previous DFW Grant E1183005. Some of the original project tasks were completed in 2012.

5.1 Problem Statement

Chemicals of potential concern (CoPC) in mine waste at the sites might migrate to surface water, sediment, soil, groundwater, and air and may be available for uptake by humans and wildlife. The impacts from release of CoPCs remain uncertain at this time. Reference concentrations for soil, surface water, and sediment are used to evaluate possible impacts to water quality, human and ecological receptors, and to quantify impacts to local creeks. Site characterization is used to characterize the mine waste, surface water, mine drainage, and provide information to support design of mitigation measures. Site characterization activities were performed under the previous Grant for this project and therefore those sampling activities are not included in this QAPP.

This project will focus on minimizing discharges to surface waters of eroded mine-wastes and contaminated drainage from the Corona Boiler House adit, Corona Drain Tunnel, and Twin Peaks adit.

5.2 Decisions or Outcomes

This project will obtain information to: complete a remediation plan to address mine drainage; prepare a plan and construct an in-situ treatment facility; design and construct a drain tunnel discharge ex-situ treatment system for any remaining discharges from the Corona Drain Tunnel; and monitor the treatment facility for loads of pollutants in Kidd Creek and James Creek.

A remediation plan will describe the nature of water quality threats and associated mitigation measures. The remediation plan is necessary to relieve the project proponent of liability under the California Water Code. Ultimately the current owner will donate portions of the Twin Peaks property to the Napa County Regional Park and Open Space District once its site and discharge contamination risks are resolved.

5.3 Water Quality Regulatory Criteria

Regulatory criteria and action levels for comparison of data are further discussed in the *Work Plan for Site Characterization for the Corona and Twin Peaks Mine Drainage Treatment Project* (Burlison, 2012b). Levels of concern in mine waste are determined in part by regulations and in part through comparison with screening benchmarks. Regulations, such as California Code of Regulations (CCR) Title 27, include prescriptive requirements for mine waste management such as capping to minimize infiltration of water, and isolation from waterways. Classification of the mine wastes in accordance with Title 27 is in part dependent on the degree of hazard they represent based on risk assessment and threats to water quality.

Evaluation of potential water quality threats relies on physical site attributes including slope, vegetation, soil characteristics, distance to receiving water, and receiving water beneficial uses. Evaluating water quality threats also requires information regarding chemistry of soil and water, both site-affected, and not affected by the site (background or reference). The appropriate scale for decision-making with respect to surface water will be determined by applicable or relevant and appropriate requirements (ARARs) and reference area comparisons. ARARs such as the Clean Water Act include chemical-specific numerical objectives to protect beneficial uses that rely on point measurement of concentrations, and other ARARs rely on numerical standards such as total maximum daily loads. Thus, the scale for decision-making will vary from a reach of a stream to points of discharge.

6.0 PROJECT/TASK DESCRIPTION

The project tasks supported under this QAPP focus on addressing environmental impacts associated with mine drainage at the Corona Mine and Twin Peaks Mine sites, in particular drainage to surface water and discharges from the adits. The ultimate goal of the project is to achieve a significant improvement in water quality through reducing metal loading to Kidd Creek. Subsurface chemical amendment, if determined to be effective at the Corona Mine, is part of this project and will identify a method to substantially reduce the metal loading from the mine tunnels and adits. The optimal method will be determined through introduction of chemicals to change the biochemical environment and prevent mobilization of metals at the source. The project tasks will support the treatment system design to address environmental issues at the mine sites. This QAPP has been prepared for sampling and analysis of surface water and biota. A separate QAPP has been prepared for aquatic biosentinel sampling and monitoring, and analysis, titled *UC Davis Mercury Bioaccumulation Monitoring for the California Bay-Delta Authority (CBDA)*, prepared by D.G. Slotton, S.M Ayers, and R.D. Weyand, dated August 15, 2008, and provided in Appendix A to this QAPP (Slotton et al., 2008).

6.1 Work Statement and Produced Products

Quantitative and qualitative data will be collected before, during, and after in-situ and ex-situ mine drainage treatment/infiltration systems are designed and constructed and operating. This information will be used to compare the treated/remediated conditions to baseline conditions and measure whether the treatment is effective. Site characterization for baseline conditions (mine waste and soil sampling), baseline surface water monitoring, and air monitoring were already conducted under the previous QAPP prepared for Grant E1183005, dated August 2012.

The tasks included in this QAPP include: (1) preconstruction studies; and (2) treatment facility monitoring and adaptive management. Aquatic biosentinel monitoring will also be conducted to assess the effectiveness of the mine remediation efforts in restoring the creek habitat for fish and aquatic insects. The results of the sampling will be reported in quarterly and final reports to DFW's Grant Manager.

Other project tasks include design of in-situ drainage treatment and ex-situ drain tunnel discharge treatment. Analytical data obtained through the monitoring tasks described below will be used to support the design of these treatment systems.

6.1.1 Preconstruction Studies

Preconstruction studies will evaluate the chemical dosing rates for the subsurface chemical amendment by determining travel times for initial, average, and peak concentrations and loads of a non-toxic tracer released at the source area and detected in Corona Drain Tunnel drainage.

The preconstruction studies include:

- Prepare a Tracer Study Plan to confirm and characterize connectivity from the subsidence feature to the Drain Tunnel Portal. This will include determining the resident time from the injection point to the exit at the drain tunnel.
- Prepare a Calibration Plan that will include pilot-scale operation methods to determine chemical dosing rates, design and operation of the full scale chemical delivery system, and drain tunnel monitoring schedule.
- Collect and submit water quality monitoring data during pilot-scale operations.

A tracer study is necessary to assess the chemical dose rate to be used in the application of subsurface chemical amendment. The tracer to be used is sulforhodamine B. This substance is non-toxic and widely used to assess potential discharges from septic systems and water treatment plants to surface water throughout California. If the tracer is not detected in Corona Drain Tunnel drainage, pilot operation of the subsurface chemical amendment system will not be performed.

The pilot scale test will include the release of chemicals (sodium hydroxide and ethanol) at rates to be determined based on results of the tracer test, and tracer concentrations measured in drainage. These chemicals will be applied at rates estimated to be sufficient to react with minerals in the drainage source area and result in consumption of the chemicals. This information will be documented in a Calibration Plan. The quantities of chemicals anticipated to be used will be small in relation to the volume of drainage discharged from the Corona Drain Tunnel, and are intended to be consumed in chemical reactions along the flow path from the remediation wells to the Corona Drain Tunnel. Thus, discharge of the chemicals to Kidd Creek at concentrations that would impact water quality will be avoided.

Drainage from the Corona Drain Tunnel will be monitored for the applied chemicals, ethanol, metals, and pH to assess the improvements to water quality due to subsurface chemical amendment. This monitoring data would also be used to confirm that no significant impact to water quality is caused by pilot operations.

6.1.2 Treatment Facility Monitoring and Adaptive Management

This task includes collecting data during and after the drainage treatment system is constructed. This will be used to compare to baseline conditions to ensure that the treatment is meeting the design objectives.

Treatment facility monitoring includes:

- Monitoring influent, effluent, and receiving water quality to characterize effectiveness of the drainage treatment system at reducing concentrations and loads of pollutants in Kidd Creek and James Creek. Monitored parameters will include: flow rates, turbidity, dissolved oxygen, pH, alkalinity, temperature, major ions (sodium and potassium), electrical conductivity, sulfate, total organic carbon, total settleable solids, total suspended solids, total mercury, methyl mercury, and trace metals (aluminum, chromium, manganese, nickel, iron and others) in filtered and unfiltered samples.
- Collecting samples monthly for one year during operation of the treatment system.

- In spring following treatment system operation, implementing a one-time biosentinel monitoring plan for comparison to 2012 baseline monitoring results. This monitoring will include collecting small fish and aquatic insects in receiving waters (whole body or multi-individual composites) and analyzing for total mercury (or methyl mercury).

Adaptive management includes verifying that the chemical doses are adequate and functioning correctly. This information will be used to develop a long-term operations, maintenance, and monitoring plan (OMMP) for the treatment facilities.

Aquatic biosentinel monitoring will be conducted by Dr. Slotton. Small fish, most commonly California roach and prickly sculpin, and aquatic insects will be collected at creek locations upstream and downstream of the Corona Mine and Twin Peaks Mine. Aquatic insects will be collected at sites where small fish are not available.

6.2 Constituents Monitored and Measurement Techniques

Table 6-1 lists the data quality objectives for the drainage treatment project under both Grants, and includes the types of sampling, the rationale (decision statement, available information), volume and extent, frequency of sample collection, and the supporting permit, as applicable. Mine waste, surface water, and aquatic biota were previously sampled in 2012 under the original Grant.

The following surface water locations were monitored in 2012 with station names (original code in parenthesis) and latitude and longitude as follows:

- Azalea Flat Creek at Mouth (140AZCKAM) 38°40'30.97"N, 122°32'59.17"W
- Bateman Creek Downstream from Mine (140BTNDFM) 38°39'47.13"N, 122°31'56.72"W
- Bateman Creek Lower Reach (140BTNLRx) 38°40'8.21"N, 122°31'44.58"W
- Bateman Creek Upper Reach (140BTNURx) 38°39'27.91"N, 122°32'9.92"W
- Bateman Creek Upstream from Mine (140BTNUFM) 38°39'44.13"N, 122°31'59.93"W
- Corona Creek Upstream from Mine (140CCKUFM) 38°40'7.05"N, 122°32'20.96"W
- Corona Flat Creek above Kidd Creek (140CFCKAK) 38°40'19.71"N, 122°32'42.15"W
- Corona Drain Tunnel Portal (140CORMLA) 38°40'15.00"N, 122°32'10.41"W
- Boiler House Adit (140CORMUA) 38°40'7.58"N, 122°32'20.64"W
- James Creek 1km Downstream (140JCK1KM) 38°40'10.46"N, 122°31'3.71"W
- Kidd Creek 200 feet below Adit (140KDC2fA) 38°40'13.41"N, 122°32'8.44"W
- Kidd Creek Upstream from Mine (140KDCUFM) 38°40'15.70"N, 122°32'10.95"W
- Kidd Creek Upstream of Oat Hill Road (140KCUOHR) 38°40'22.98"N, 122°32'25.65"W
- McNabb Creek at Mouth (140MNCRAM) 38°40'21.96"N, 122°32'46.15"W
- Twin Peak Seep into Bateman Creek (140TPSIBC) 38°39'42.79"N, 122°32'0.20"W
- Twin Peaks Adit (140TPKADR) 38°39'44.37"N, 122°32'4.76"W

The following locations were sampled for fish and aquatic insects in 2012 and analyzed for mercury to establish baseline conditions (Slotton, 2013).

<u>Site Description</u>	<u>Sampling Date</u>	<u>GPS North</u>	<u>GPS West</u>	<u>Relative Location</u>
Bateman Creek abv Twin Peaks Mine	5/3/12	38.66152	-122.53346	above Twin Peaks Mine
Bateman Creek blw Twin Peaks Mine	5/3/12	38.66574	-122.53069	below Twin Peaks Mine
Kidd Creek abv Corona Mine	5/7/12	38.67203	-122.53860	above Corona Mine
James Creek at Aetna Road	5/8/12	38.66928	-122.51872	app. 1 mile blw mines
James Creek blw Udnick Creek	5/9/12	38.67195	-122.47679	app. 3 miles blw mines
Udnick Creek	5/9/12	38.67853	-122.45182	un-mined control creek
Pope Creek at Barnett Rd	5/11/12	38.64327	-122.42028	app. 7 miles blw mines
Swartz Ck at Aetna Springs Rd	5/19/12	38.65333	-122.47640	mined control ck (blw Aetna)

Under this current Grant, surface water, adit drainage, fish and aquatic insects will be collected to assess the treatment effectiveness.

Table 6-1. Data Quality Objectives (DQO) of Corona and Twin Peaks Mercury Mines Project, Napa County, California

TARGET POPULATION (CONSTITUENT)	DECISION STATEMENT	AVAILABLE INFORMATION REFERENCE	VOLUME AND EXTENT	METALS CONCENTRATION	CHEMICAL PROPERTIES	PHYSICAL PROPERTIES	STORM EFFECTS	SUPPORTS
Mine Waste: Waste Rock	<ul style="list-style-type: none"> If the mine waste contains CoPCs above concentrations that would require excavation and/or isolation from the environment, then appropriate next steps will be identified. If the mine waste does not contain CoPCs at concentrations that would require excavation and/or and isolation from the environment, then enhanced revegetation would be implemented. 	Slowey, 2007 USGS OFR 1132, 2007 Tuleyome, 2013	<ul style="list-style-type: none"> Site mapping 	Minimum of 1 Incremental Sample Method composite per pile Corona: 3 piles Twin Peaks: 1 pile			Minimum of two samples for analysis per pile during runoff	Water Quality Evaluation Risk Evaluation
Mine Waste: Tailings								
Mine Drainage	<ul style="list-style-type: none"> If the drainage does not pose an unacceptable threat to water quality then the existing treatment system will be improved. If the drainage poses an unacceptable threat to water quality, then appropriate next steps will be identified. 	CLS, 1997 MFG, 2002 Slowey, 2007 USGS OFR 1132, 2007 Tuleyome, 2013	<ul style="list-style-type: none"> Site mapping 	Quarterly to Monthly Sampling and analysis	Quarterly to Monthly Sampling and analysis	(Total Suspended Solids, Total Dissolved Solids)	Minimum of two samples for analysis per pile during runoff	Water Quality Evaluation Risk Evaluation
	<ul style="list-style-type: none"> If the drainage from the Corona Drain Tunnel can be efficiently treated by subsurface chemical amendment to prevent water quality impacts, then a subsurface chemical amendment dosing system will be designed, built, and operated. If drainage from the Corona Drain Tunnel cannot be efficiently treated by subsurface chemical amendment to prevent water quality impacts, then an ex-situ treatment system will be designed, built, and operated. 		<ul style="list-style-type: none"> Flow measurement Pilot Test Subsurface Chemical Amendment System Infiltration Testing Monthly sampling and testing	Quarterly to Monthly Sampling and analysis	Quarterly to Monthly Sampling and analysis	(Total Suspended Solids, Total Dissolved Solids)	Minimum of two samples for analysis per pile during runoff	System Design Water Quality Evaluation Risk Evaluation
	<ul style="list-style-type: none"> If drainage from mine waste is not present, then it does not degrade water quality. If drainage from mine waste is present and does not degrade water quality then additional actions are not necessary. If drainage from mine waste is present and degrades water quality, then appropriate next steps will be identified. 		<ul style="list-style-type: none"> Site mapping Public Domain Information On-Site Measurement 	Quarterly to Monthly Sampling and analysis	Quarterly to Monthly Sampling and analysis	Not Applicable	Minimum of two samples for analysis per pile during runoff	Water Quality Evaluation Risk Evaluation
Surface Water and Storm Water	<ul style="list-style-type: none"> If CoPC concentrations are at or above levels of concern in surface water, then monitoring to assess treatment system effectiveness will be implemented. If CoPC concentrations are below levels of concern in surface water, then additional monitoring may not be necessary. 	CLS, 1997 MFG, 2002 Slowey, 2007 USGS OFR 1132, 2007 Tuleyome, 2013	<ul style="list-style-type: none"> Site mapping Public Domain Information On-Site Measurement 	Quarterly to Monthly Sampling and analysis	Quarterly to Monthly Sampling and analysis	As specified in Stormwater Pollution Prevention Plan (SWPPP)	Surface Water: Monthly samples desirable, minimum of 12; Storm water: Minimum of 2 sample events during runoff per year	Water Quality Evaluation Risk Evaluation
Site Soil	<ul style="list-style-type: none"> If CoPCs in soil pose an unacceptable risk to receptors, then appropriate next steps will be identified. If CoPCs in soil do not pose an unacceptable risk to receptors, then no action is necessary. 	Slowey, 2007 USGS OFR 1132, 2007	<ul style="list-style-type: none"> Site Mapping 	Assume similar to or lower than mine waste Assume small volume of in place soil associated with contaminant sources is potentially contaminated, to be addressed same as for mine waste			Not applicable	Not Applicable
Reference Soil and Surface Water	<ul style="list-style-type: none"> If suitable reference areas for comparison of CoPC concentrations in soil and surface water with Site affected media are not available, then reference concentrations cannot be identified, and alternative sources of reference concentrations (regional, local, etc.) need to be evaluated. If suitable reference areas for comparison of CoPC concentrations in soil and surface water with Site affected media are available, then reference concentrations can be identified for use in evaluating risks. 	Slowey, 2007 USGS OFR 1132, 2007	<ul style="list-style-type: none"> Not applicable, reference area will be identified on maps. 	Soil: Minimum of 4 Composite/Incremental Sampling Method representing soil types present Water: Minimum of one upstream sample per sampling event				Water quality evaluation Risk evaluation

TARGET POPULATION (CONSTITUENT)	DECISION STATEMENT	AVAILABLE INFORMATION REFERENCE	VOLUME AND EXTENT	METALS CONCENTRATION	CHEMICAL PROPERTIES	PHYSICAL PROPERTIES	STORM EFFECTS	SUPPORTS
Air	<ul style="list-style-type: none"> If CoPCs are migrating through air at levels that pose an unacceptable risk to receptors, then appropriate next steps will be identified. If CoPCs migrating through air do not pose unacceptable risks to receptors, then no action is necessary. 	EPA Acute Exposure Guideline Levels (AEGLs), 2012	<ul style="list-style-type: none"> Visual Observation Particulate Measurement Vapor measurement 	Vapor measurement and Modeling	From literature	From literature	Vapor measurement	Risk evaluation

Notes:
CoPC = Chemical of Potential Concern

6.3 Project Schedule

Table 6-2 includes the schedule for activities performed under the QAPP.

Table 6-2 Project Schedule and Deliverables

GRANT PROJECT TASK	SUB-TASKS	GRANT PRODUCT	ESTIMATED INITIATION DATE OR TIMEFRAME	ESTIMATED COMPLETION DATE OR TIMEFRAME
Task 7 Preconstruction Studies	Tracer Study	Tracer Study Plan	March 2016	April 2016
	Pilot Scale	Calibration Plan	Spring 2016	June 2016
	Water Quality Monitoring at Drain Tunnel	Water quality monitoring data	During pilot-scale operations	Completion of Pilot Scale operation
Task 10 Treatment Facility Monitoring and Adaptive Management	Monitor Treatment Facility and Creeks during Operation	Water Quality Monitoring Report (Monthly data on influent, effluent, and receiving waters)	During operation of treatment system	Summer 2018
	Biosentinel Monitoring	Biosentinel Monitoring Report	Spring after treatment system operation	Due with Final Report November 30, 2018

6.4 Geographical Setting

The project area is located in the James Creek sub watershed, tributary to Pope Creek, a major tributary to Lake Berryessa. Discharges from Lake Berryessa pass through Putah Creek to the Yolo Bypass in the northern Delta. Approximately 40 more abandoned mines exist in this region, known as the East Mayacmas Mining District. Approximately half of the East Mayacmas and Clear Lake Mining Districts and all of the Knoxville Mining District lie within the Putah Creek Watershed, cumulatively representing the third largest mercury producing region in the nation when production was most active between 1850 and 1961 (US Department of Interior, Bureau of Mines [USBM], 1965). Half of all toxic mercury pollution that enters the Sacramento River watershed comes from the Putah and Cache Creek watersheds.

This mining legacy contributes to the state's listing as impaired of James Creek (nickel and mercury), Lake Berryessa (mercury), and lower Putah Creek (mercury and boron). James Creek has been identified as prime trout habitat. A fish consumption advisory is posted for Lake Berryessa and for lower Putah Creek because of fish mercury contamination. Lower Putah Creek is a Wild Trout stream and drains into the Yolo Bypass, a nationally recognized fish rearing,

wildlife habitat, farming, and flood control area with some of the highest mercury concentrations in the Bay-Delta.

6.5 Constraints

Continuous discharges of drainage water from three adits at the Corona and Twin Peaks Mines are acidic with high concentrations of iron and nickel, and some mercury in the suspended solids. The Twin Peaks and the Corona adits have been improved to the point where they no longer discharge into the creek. However, the current improvements are not ideal—difficult to maintain and clogging with iron precipitate. This project will develop and implement a reliable, long-term, maintainable solution for both dissolved nickel and for precipitated solids.

The Corona Drain Tunnel's discharge has not been controlled to date. The adit is located at the bottom of a steep grade in a narrow canyon. Site improvements will be required to allow reasonable access for construction, operations and maintenance. In situ treatment/infiltration will be implemented to attempt to prevent untreated discharge to Kidd Creek. Additional ex-situ may be required to address any remaining chemicals of potential concern (CoPCs) not treated using the in-situ treatment. The CoPCs are aluminum, chromium, manganese, mercury, nickel, and iron. The full list of metals to be analyzed includes: aluminum, antimony, arsenic, barium, beryllium, boron, cadmium, calcium, cobalt, chromium, copper, iron, magnesium, manganese, molybdenum, mercury, methyl mercury, nickel, lead, potassium, selenium, silver, sodium, thallium, vanadium, and zinc.

Each of the three mine adits on the property discharges elevated concentrations of some CoPCs. Construction of the treatment/infiltration systems will include site preparation, water conveyance, and system construction. Demonstrating effective cleanup methods is critical to addressing this toxic health hazard. The testable hypothesis is that in-situ and ex-situ treatment systems will provide long-term treatment of adit drainage to improve downstream water quality.

7.0 QUALITY OBJECTIVES AND CRITERIA FOR MEASUREMENT DATA

This section presents the QA objectives for the project data quality indicators: precision, accuracy, representativeness, completeness, detection limits, and comparability. Tables 7-1 through 7-3 summarize the analytical parameters and laboratory QA/QC objectives for each analysis. Fish and aquatic insects will be collected and analyzed for methyl mercury or total mercury in accordance with the UC Davis Mercury Bioaccumulation Monitoring for the California Bay-Delta Authority QAPP (Appendix A). No soil data will be collected. Soil data was previously collected under the site characterization and QAPP for Grant E1183005.

7.1 Precision and Accuracy

Precision and accuracy goals depend on the types of samples, analyses, and ultimate use of the analytical data. Accuracy will be evaluated from matrix spike/matrix spike duplicate (MS/MSD) samples as percent recovery (%Recovery)). In addition, laboratory control samples (LCS) prepared from a different stock solution than the calibration standards and traceable to established standards will be analyzed with each sample batch and the % Recovery calculated. Precision will be evaluated as the relative percent difference (RPD) between MS/MSD results. Precision also will be evaluated using laboratory duplicates. An RPD will be calculated for each analyte in the duplicate pair. The QA objectives for all analyses will be 25% RPD for precision and 75-125 % for accuracy.

7.2 Detection Limits

Expected detection limits listed in the tables below are based on historical method detection limit (MDL) studies by the laboratory and experience with the type of matrix being analyzed within the evaluation. Method blank results will be reported only at concentrations greater than the detection limit to decrease reporting of low-level blank contamination.

7.3 Completeness

Completeness is an assessment of the amount of valid data obtained from a measurement system compared to the amount of data expected. The percent completeness is calculated as follows: the number of samples yielding acceptable data is divided by the total number of samples collected multiplied by 100. The objective for the degree of completeness is 90 percent. If completeness is less than 90 percent, this will be documented during the data quality assessment why this objective was not met and the impact of any lower percentage on the project.

7.4 Representativeness

For this project, representativeness involves sample size, sample volume, and sampling locations. The QA goal is to obtain an adequate number of samples that represent the media and its properties at the time of collection. The volume of sample collected also depends on the analytical method chosen, allowing for QC sample analysis and re-analysis if needed. Method blanks are also an indicator of representativeness. If target compounds are not detected in

method blank samples, then target compounds detected in analytical samples are representative of the sample rather than laboratory or cross-contamination.

7.5 Comparability

Data comparability will be maximized by using standard EPA analytical methods when possible. Procedures for all planned methods are specified, and any deviations from the methods will be documented. All results will be reported down to the detection limit in the standard units shown in the tables or in the units specified in the method. Comparability also will be maximized by use of consistent sample collection techniques and analytical methods.

Table 7-1 Aqueous Metal Detection Limits and Screening Criteria

ANALYTE	WATER (TOTAL AND DISSOLVED)		
	Screening Criteria (µg/L) ¹	Analytical Detection Limit (µg/L)	Analytical Method ³
Antimony	6	1.5	EPA 200.8/3010/SW-846 6020A
Arsenic	10	2.0	EPA 200.8/3010/SW-846 6020A
Aluminum	50	0.1	EPA 200.8/3010 /W-846 6020A
Barium	1,000	2.0	EPA 200.8/3010/SW-846 6020A
Beryllium	4	0.2	EPA 200.8/3010/SW-846 6020A
Boron	1,000	0.2	EPA 200.8/3010/SW-846 6020A
Cadmium	1.9 ²	0.2	EPA 200.8/3010/ SW-846 6020A
Calcium	NA	100	EPA 200.8/3010/ SW-846 6020A
Cobalt	50	0.2	EPA 200.8/3010/ SW-846 6020A
Total Chromium	50	1.5	EPA 200.8/3010/ SW-846 6020A
Copper	6.9 ²	0.4	EPA 200.8/3010/ SW-846 6020A
Iron	300	60.0	EPA 200.8/3010/ SW-846 6020A
Magnesium	NA	20	EPA 200.8/3010/ SW-846 6020A
Manganese	50	4.0	EPA 200.8/3010/ SW-846 6020A
Mercury (Total)	0.05	0.0005	EPA 1631/1669
Methyl Mercury (Total)	0.7	0.00005	EPA 1630/1669
Molybdenum	10	0.7	EPA 200.8/3010/ SW-846 6020A
Nickel	39 ²	0.7	EPA 200.8/3010/ SW-846 6020A
Lead	2.0 ²	0.3	EPA 200.8/3010/ SW-846 6020A
Potassium	NA	20	EPA 200.8/3010/ SW-846 6020A
Selenium	50	0.5	EPA 200.8/3010/ SW-846 6020A
Silver	2.2 ²	0.1	EPA 200.8/3010/ SW-846 6020A
Sodium	NA	20	EPA 200.8/3010/ SW-846 6020A
Thallium	1.7	0.3	EPA 200.8/3010/ SW-846 6020A
Vanadium	50	1.5	EPA 200.8/3010/ SW-846 6020A
Zinc	89 ²	10.0	EPA 200.8/3010/ SW-846 6020A

Notes:

µg/L = micrograms per liter

1. Compilation of Water Quality Goals, California Regional Water Quality Control Board—Central Valley Region (August 2007)
2. Varies with hardness for protection of fresh water aquatic life, value provided is for 70 mg/L hardness.
3. Method 6010/200.7 (ICP) or GFAA may be substituted if detection limits are met.

Table 7-2 Field Analytical Methods and Detection Limits

PARAMETER	PHASE	METHOD	DETECTION LIMIT	UNITS
Tracer (sulforhodamine B)	Total	Fluorometry (or Portable Data Logger)	1.0	µg/L
Dissolved Oxygen (DO)	Total	DO Polarographic Probe	0.0 to 19.9	mg/L or (% Saturation)
pH	Total	EPA 150.1	0.1 (0 to 14)	pH
Specific Conductance	Total	EPA 120.1	1	µmho
Oxidation Reduction Potential (ORP)	Total	SM 2580 AB	± 25mv	mv as Eh

mg/L = milligrams per liter, ng or mg/m³ = nanograms or milligrams per cubic meter, mv = millivolts, ORP = oxidation reduction potential

Table 7-3 Laboratory Measurement Quality Objectives for Analytical Parameters for Water

ANALYTE	ANALYTICAL METHOD OR EQUIVALENT	WATER DETECTION LIMIT ^A	PRECISION (RPD) ^B	ACCURACY (% R) ^C
Total and Dissolved Metals	EPA 200.8	Various (see Table 7-1)	25	75-125
Total Mercury	EPA 1631B & E	0.5 ng/L	25	75-125
Methyl Mercury	EPA 1630/1669	0.05 ng/L	25	70-130
Total Organic Carbon	EPA 415.3/SM5310B	0.02 mg/L	25	75-125
Hardness (as CaCO ₃)	SM2340/EPA 200.8/EPA 6010	1 mg/L	25	75-125
Chloride	EPA 300.1	20 µg/L	25	75-125
Nitrate-N	EPA 300.1	400 µg/L	25	75-125
Turbidity	EPA 180.1	0.1 NTU	25	75-125
Sulfate, Chloride, Nitrate	EPA 300.0	200 µg/L	25	75-125
Alkalinity as CaCO ₃	EPA 310.1/SM2320B	1.0 mg/L	25	75-125
Dissolved Oxygen	EPA 360.1	1 mg/L	10	5% bias
pH	EPA 150.1	0.1 pH units	10	0.1 % bias
TDS/TSS	EPA 160.1/160.2	10 mg/L / 4 mg/L	10	5% bias
ORP	SM 2580 AB	± 25 mv as Eh	10	5% bias
SC	EPA 120.1	1 µmho	10	5% bias
Ethanol	EPA 8015B	2.0 mg/L (MDL 0.15 mg/L)	10	75-125

Notes:

a = Detection limits may be higher for samples with elevated contaminant concentrations.

b = Precision as relative percent difference (RPD)

c = Accuracy as % R of matrix spikes

ORP Oxidation Reduction Potential

mv millivolts

MDL method detection limit

SC Specific Conductance

%R Percent recovery

RPD Relative percent difference

SW-846 Test Methods for Evaluating Solid Waste/Chemical Methods (EPA 1997)

SM Standard Methods for Chemical Analysis of Water and Wastes (EPA 1983)

mg/L Milligrams per liter

meq/L Milliequivalents per liter

mg/kg Milligrams per kilogram

µg/L Micrograms per liter

ng/L Nanograms per liter

ng/g Nanograms per gram

NTU Nephelometric Turbidity Units

8.0 SPECIAL TRAINING NEEDS/CERTIFICATION

8.1 Specialized Training or Certifications

Training requirements specified in the Health and Safety Plan (HSP) (Burleson, 2016) will be completed as required for field sampling. Pilot scale monitoring of tracer chemicals will be conducted in the field accordance with the instrument user's manuals.

In addition, personnel responsible for operating mechanical equipment will receive the necessary operating instruction on that equipment. Sampling personnel will be trained to use industry-standard practices.

8.2 Training and Certification Documentation

All personnel involved in field activities will be familiar with the requirements of this project QAPP. Hard copies of the current approved version of the project-specific QAPP and its appendices will be maintained by QAPP Personnel for ready reference. An electronic file will be available in the project team's online Dropbox site.

Each analytical laboratory maintains their training documentation and certification.

8.3 Training Personnel

Sampling oversight will be provided by Greg Reller and Tim Tsukamoto. Peter Green will be responsible for UC Davis laboratory's processing and analysis of the water samples. Darrell Slotton will be responsible for the biota sampling and analysis.

9.0 DOCUMENTS AND RECORDS

9.1 Field Documentation

Sample collection activities will be documented in permanently bound, page-numbered, weather-resistant field logbooks assigned to each field sampling technician or coordinator. Each notebook will be identified by project monitoring activity and the individual assigned custody of the logbook. If logbook custody is transferred to another individual, such transfer will be noted in the logbook and signed and dated by both parties. All entries will be made in indelible ink; errors will be corrected by one single line through the text being revised, and all such corrections will be initialed and dated.

Bound field logbooks will be used to record the following information:

- Sample date, time, types, numbers, and quantities;
- Sample container preservation steps performed;
- Sample locations or station identification (ID) number or code;
- Sampling equipment used;
- Decontamination steps performed;
- Calibration and maintenance actions;
- Information identifying other field equipment by equipment identification number; model/manufacturer, serial number, or other unique identifier; and
- Confirmation that Chain-of-Custody (COC) forms or notebook pages were properly completed and sample custody transferred as required by this QAPP.

In addition, other ancillary information should be recorded on a field form, including:

- Summary of daily activities;
- Any deviations from the associated work plan, sampling plan, QAPP, or standard operating procedure (SOP);
- Time of arrivals/departures of field technician and/or other visitors to the sampling station;
- Weather conditions;
- Time and subject of any incoming or outgoing telephone/radio contacts; and
- Any unusual events.

The Surface Water Ambient Monitoring Program (SWAMP) data management plan (SWAMP, 2015) provides a list of general information collected in the field and added to the electronic deliverable data (EDD), to maintain consistency regarding samples collected and analyzed, and includes the following fields (if applicable):

- Lab sampled ID (provided by laboratory)
- Station Code
- Event Code
- Protocol Code
- Location Code
- Sample Date
- Collection Time
- Collection Method Code
- Sample Type Code
- Replicate
- Collection Depth
- Unit collection depth
- Project Code
- Agency Code

The SWAMP Data Management Plan provides definitions for the fields listed above, and field data sheet examples, as well as Excel templates for filling out the field information.

Analytical results in SWAMP format will include the above field collected information along with:

- Analyte name
- Units
- Dilution factor
- Result
- Result qualification code
- Method detection limit
- Reporting limit
- QA code
- Expected value (if applicable)
- Lab result comments (if any provided).

The SWAMP Data Management Plan provides example Excel templates for recording field information and reporting analytical chemistry results. The plan also provides detailed instructions on how to submit data to the SWAMP Data Management Team, which is through the Online Data Checker website. Data is rejected and returned if the report is incomplete or naming conventions are not followed.

9.2 Chain-of-Custody Records

During sampling activities, a “paper trail” of sample custody must be maintained from the time the samples are collected until laboratory data are issued. Initial information concerning sample collection will be recorded in the field logbook. Information on the custody, transfer, handling, and shipping of samples will be recorded by the field technician on a COC form provided by the destination laboratory. A COC form or equivalent notebook page will be completed for each set of samples collected daily and will contain the following information:

- Field Technician’s signature;
- Project name;
- Date and time of collection;
- Sample identification number and matrix;
- Analyses requested;
- Number of containers;
- Signature of persons relinquishing custody, dates, and times;
- Signature of persons accepting custody, dates, and times;
- Method of shipment; and
- Shipping papers/waybill identification number (as appropriate).

A copy of each as-transmitted COC form or equivalent log-book or forms will be retained in the monitoring program records.

9.3 Analytical Laboratory Records

The laboratories will be responsible for preparing analytical laboratory reports and electronic data deliverables (EDDs) that are reviewed and approved by the laboratory’s QA Officer. All surface water samples will be reported using the SWAMP EDD format. The SWAMP program requires reporting of accuracy, precision, and blank QC samples. Example EDDs and field forms are provided through the SWAMP Data Management Plan program.

A written report will be prepared by the analytical laboratory documenting all the activities associated with each sample analysis. At a minimum, the following will be included in this report:

- Results of the laboratory analysis and laboratory QA/QC results;
- All protocols used during analyses;
- COC procedures; and
- Discussion of any deviations from the approved methods.

The EDDs will be obtained for all analytical results in SWAMP format. The EDD will correspond exactly to the hard copy data.

9.4 Monitoring Program Record Files

Site monitoring program records are defined as completed, legible documents, which furnish objective evidence of the quality and completeness of the data acquired pursuant to the requirements of the project activities. These records will be organized, filed and maintained under the direction the QA Officer, and will include, at a minimum:

- Copies of all bound field logbooks;
- Field copies and original (laboratory) copies of all COC forms;
- Personnel training records;
- Incoming and outgoing correspondence related to the project activity (e.g., letters, telephone conversation records, faxes, and hard copies of e-mail messages);
- Copies of all laboratory agreements and amendments thereto;
- As-received laboratory reports and data packages;
- All approved field changes;
- Draft and final versions of all reports and any associated laboratory data packages;
- Corrective and preventive action documentation or forms;
- Assessment and/or technical review reports;
- Data validation reports; and
- Draft and final versions of this QAPP and its appendices.

9.5 Field Change Request Forms

Field sampling activities can experience unexpected situations that will require deviations or modifications to the requirements of the QAPP (and/or sampling plan). Other changes may be required by other external stakeholders during the course of the project and monitoring program. The QA Officer or Project Manager may authorize the field coordinator to undertake modifications to the QAPP or its appendices provided that the scope of such modifications is discussed with and approved by the QA Officer.

Group B Data Generation and Acquisition

10.0 SAMPLING PROCESS DESIGN

The sampling process will include a combination of judgmental and probability-based (statistical) design depending on the objectives and constituents sampled for the tasks described in Chapter 6, which are: pre-construction studies (tracer and pilot-scale) and treatment facility monitoring and adaptive management. Discharge samples and surface water monitoring samples will be collected in accordance with information listed in Table 10-1.

10.1 Pre-Construction Studies (Tracer and Pilot-scale Operations)

Tracer studies and pilot-scale operations are utilized to provide a longer-term improvement in water quality by designing a method to precipitate metals, and impede oxidation of the sulfide minerals in the mine discharge. The goal is to prevent metal mobilization by amending the subsurface chemical environment. The tracer chemical will be released above the Corona Drain Tunnel into the mine and measured in the portal outfall. If the tracer chemical is detected in the outfall discharge within about one day, then an organic lipid, such as ethanol will be added to subsurface mine and used to stimulate growth of sulfate-reducing bacteria within the mine. If reducing conditions are established within the mine, the metals will be removed by sulfate reduction and sulfide precipitation. If reducing conditions are not established using bacteria, then the pH will be lowered using sodium hydroxide. Therefore, both ethanol and pH (to measure sodium hydroxide) will be measured in the drain tunnel discharge to evaluate the effectiveness of the in-situ treatment.

Sampling for the tracer study and pilot-scale operations will be performed by TKT and includes collecting sufficient water from the Drain Tunnel drainages evaluating the effectiveness of bench-scale tracer detection and infiltration treatment (using ethanol or sodium hydroxide) and later to determine the chemical dosing rates. TKT will complete a Tracer Study Plan that will detail the study design to confirm and characterize the connectivity from the subsidence feature to the Drain Tunnel portal and determine the residence time from the injection point to when it exist the drain tunnel. The pilot-scale methods will be described in a Calibration Plan that will explain the chemical dosing rates, design and operation of the full scale chemical delivery system, and the drain tunnel discharge monitoring schedule. Tracer chemicals and ethanol will be sampled at the drain tunnel. These samples would facilitate a judgmental design because they will be collected directly from the Drain Tunnel drainage. Additional surface water samples collected by Peter Green for the water quality monitoring will supplement the data analyzed for this task.

10.2 Treatment Facility Monitoring and Adaptive Management (Surface Water and Aquatic Biosentinel Monitoring)

The sampling design for the effectiveness and post-treatment monitoring task is judgmental. Monitored parameters include flow rates, turbidity, dissolved oxygen, pH, alkalinity, temperature, conductivity, major cations (calcium, magnesium, potassium, and sodium),

hardness, sulfate, nitrate, chloride, total organic carbon, total dissolved solids and total suspended solids, total and methyl mercury, and trace metals in both filtered and unfiltered samples. The complete metals list includes: aluminum, antimony, arsenic, barium, beryllium, boron, cadmium, calcium, cobalt, chromium, copper, iron, magnesium, manganese, mercury, methyl mercury, molybdenum, nickel, lead, potassium, selenium, silver, sodium, thallium, vanadium, and zinc. Selected metals (chemicals of concern include aluminum, chromium, iron, manganese, and nickel) will be analyzed more frequently. The monitoring will be conducted to determine what metals are being mobilized (and their concentrations) compared to the baseline conditions in the creeks and adits, and whether extreme chemical conditions, such as elevated pH, have been created.

Table 10-1 provides the proposed sampling locations and analytical parameters. General field parameters will be measured as needed at all monitoring stations, when water is present, in accordance with the frequency listed in Table 10-1. Water samples for dissolved metals will be filtered in the field through a 0.45 micron filter. Metals analysis will include total and dissolved metals (on filtered samples). Mercury will be analyzed on unfiltered samples only.

Grab samples will be collected monthly or quarterly spanning the yearly cycle during operation of the treatment system from the proposed monitoring stations at the upstream and downstream locations within the creeks and adits (as needed). Discharges and receiving water (upstream and downstream) will be sampled to characterize the effectiveness of the drainage treatment system at reducing the concentrations and loads in Bateman, Kidd, and James creeks. The full metal list or only the COPCs (aluminum, chromium, iron, manganese, mercury, and nickel) will be analyzed at the proposed frequency listed in Table 10-1.

Aquatic biosentinel monitoring will consist of collecting small fish and aquatic insects in the spring following treatment system operation for comparison to 2012 baseline monitoring results. The small fish, most commonly California roach and prickly sculpin, and aquatic insects will be collected at creek locations upstream and downstream of the Corona Mine and Twin Peaks Mine. The small fish will be prepared and analyzed individually, whole body, or as multi-individual composites as appropriate, and analyzed for methyl mercury. Aquatic insects will be collected at sites where small fish are not available. The aquatic insects will be prepared as multi-individual composites of whole individuals (sample size will be comprised of eight or more individuals) collected in 3 to 4 replicates at each sampling site.

Table 10-1. Field Sampling Locations and Analytical Methods

Water	Sample Frequency Based on Available Flow and Grant Years	Approximate Number of Field Samples									
		Total Metals/Dissolved Metals (Full List) ¹	Selected Metals (Al, Cr, Fe, Mn, Ni)	Total Mercury (low level)	Total Methyl Mercury (low level)	TOC or Ethanol (as applicable)	Sulfate, Chloride, Nitrate	Major Cations (CA, Mg, Na, K)	Alkalinity, TDS, TSS, Turbidity, Hardness	Tracer Chemical	Field Temperature, pH, DO, ORP, SC
Surface Water (see station locations)											
Kidd Creek upstream from Oat Hill Road	Biannual – when flowing	3	0	1	1	0	3	3	3	0	3
Kidd Creek upstream from Drain Tunnel	Quarterly and monthly after remediation starts – when flowing	6	12	3	3	12	12	6	6	0	12
Kidd Creek 200 Feet downstream from Drain Tunnel	Quarterly and monthly after remediation starts	6	12	3	3	12	12	6	6	12	12
Bateman Creek upstream from Twin Peaks Mine	Quarterly – when flowing	4	0	2	2	0	4	4	4	0	4
Bateman Creek downstream from Twin Peaks Mine or other downstream access point	Quarterly – when flowing	4	0	2	2	0	4	4	4	0	4
James Creek 1 km downstream	Quarterly and monthly after remediation starts	3	12	1	1	0	3	3	3	0	3
Twin Peaks Seep into Bateman Creek	Quarterly – when flowing	2	0	1	1	0	2	2	2	0	2
Mine Drainage (see station locations)											
Boiler House Adit	Quarterly and monthly after remediation starts, with additional sampling during tracer test and pilot subsurface chemical amendment trial.	8	12	4	4	12	12	8	8	12	12
Corona Drain Tunnel Portal	Quarterly and monthly after remediation starts, with additional sampling during tracer test and pilot subsurface chemical amendment trial.	8	12	4	4	12	12	8	8	12	12
Twin Peaks Adit	Quarterly	8	6	1	1	0	6	6	6	0	6
Twin Peaks Adit infiltration trench	Quarterly	0	6	0	0	0	6	6	6	0	6
Twin Peaks Adit settling basin (following construction)	Quarterly	0	6	0	0	0	6	6	6	0	6
Boiler House Adit infiltration trench	Quarterly	0	6	0	0	0	6	6	6	0	6
Boiler House Adit settling basin (following construction)	Quarterly	0	6	0	0	0	6	6	6	0	6
Drain Tunnel Portal settling basin (following construction)	Quarterly	0	6	0	0	0	6	6	6	0	6
Field Quality Control Samples											
Duplicate	10% of field samples per event/each event	8	12	4	4	NA	12	8	8	NA	NA
Matrix Spike/Matrix Spike Duplicate	10% of field samples per event/each event	8	12	4	4	NA	12	8	8	NA	NA
Field Blank/Filter	Each sampling event	Yes	NA	Yes	Yes	NA	NA	NA	NA	NA	NA

Notes: NA = not applicable

¹ The full metals list includes: aluminum, antimony, arsenic, barium, beryllium, boron, cadmium, calcium, cobalt, chromium, copper, iron, magnesium, manganese, mercury, molybdenum, nickel, lead, potassium, selenium, silver, sodium, thallium, vanadium, and zinc.

11.0 SAMPLING METHOD

11.1 Field Sampling Techniques

11.1.1 Surface Water Field Measurements

Field parameter measurements will be made in-situ in the streams or drainage, whenever possible. If an in-situ measurement is not possible, then the measurement will be made streamside by collecting the water using a decontaminated plastic container. Field equipment and meters will be calibrated prior to each sampling event, and when an instrument sampling probe is changed, in accordance with manufacturer's instructions. The sampling probe will be decontaminated after each measurement, and before each sample is measured to avoid cross contamination. Measurements will be recorded on field sampling forms and in field logbooks.

11.1.2 Fish and Aquatic Insect Sampling

Field sampling and handling techniques for fish and invertebrates will be collected according to the UC Davis SOP #1, included in the *UC Davis Mercury Bioaccumulation Monitoring for the California Bay Delta Authority (CBDA) QAPP* (Slotton, D.G. et al., 2008) (Appendix A).

11.1.3 Mine Drainage and Surface Water Sampling

Mine drainage and surface water samples will be collected by immersion of sample containers. Pre-cleaned sample containers provided by the laboratory will be used to collect surface water samples. Surface water will be collected by immersing sample containers directly into the water to be sampled when and where sufficient water is present. The mouth of the sample container will face upstream, and the sample will be collected at the approximate middle of the water column at the sampling location. Care will be taken to avoid collecting water with disturbed sediment by collecting samples upstream of sampling personnel positions and progressing from downstream to upstream sample locations. Samples for dissolved constituents will be collected by pumping water directly from the surface water and through a 0.45 micron disposable filter prior to filling the sample container. Preserved sample containers will be filled without overflowing to avoid diluting preservatives. Unpreserved containers will be rinsed two times with water from the sample location prior to collecting the sample when sufficient water is present. At locations where sufficient water is not present to immerse the sample container, each container will be filled at a location where the natural flow of water allows, such as flow over the lip of a pool. Sample container rinsing will not be performed at low-flow locations.

Surface water samples collected for low level methyl-mercury (Method 1630) will be collected using the EPA Method 1669, *Sampling Ambient Water for Determination of Trace Metals at EPA Water Quality Criteria Levels* (EPA, 1998), described in Section 11.2.

11.2 Decontamination

Sampling equipment will be triple rinsed prior to taking each sample. All rinsate may be disposed of on-site. Field personnel will handle field equipment and containers carefully to minimize the potential for cross-contamination. Equipment (filter) method blanks will be collected with filtered samples.

EPA Method 1669, *Sampling Ambient Water for Determination of Trace Metals at EPA Water Quality Criteria Levels* (EPA, 1998) is necessary to preclude contamination during the sampling process. Before samples are collected, all sampling equipment and sample containers are cleaned in a laboratory or cleaning facility using detergent, mineral acids, and reagent water, and filled with weak acid and double bagged for shipment. The laboratory is responsible for generating an acceptable equipment blank to demonstrate that the sampling equipment and containers are free from trace metals contamination before they are shipped to the field sampling team. Field blanks are also required to be collected. Upon arrival at the site, a two-person team (clean and dirty hands) collects the samples. Clean hands are used for contact with the sample bottles. Dirty hands for operating equipment. All personnel must wear gloves, and sampling equipment used must be non-metallic. Field duplicate samples are also required.

11.3 Sample Containerization, Preservation, and Holding Times

Table 11-1 presents containerization, preservation, and holding time requirements for each parameter to be analyzed by the laboratory. Container sizes have been selected to allow for adequate sample volume for the required analysis. All containers will be obtained from the laboratory, and the laboratory will place the appropriate amount of preservatives in each container before shipment. Holding times presented are identified from the analytical methods. The holding time is the duration a sample can be held—from the time it is collected to the time it is analyzed—and still produce acceptable results.

Table 11-1. Analytical Laboratory Sample Analysis, Volumes, Holding Times, and Preservation Requirements

ANALYSIS	METHOD	MATRIX	SAMPLE VOLUME REQUIRED	HOLDING TIME	PRESERVATION
Total and Dissolved Metals ^a	EPA 200.8/SW-846 6020A(ICP/MS)	Water	500 Milliliter poly bottle	180 days	Nitric Acid pH<2 Cool to 4 °C
Total Mercury	EPA 1631E/1669	Water	500 ml glass	If unpreserved, 48 hr	Cool to 4 °C
Total Methyl Mercury	EPA 1630/1669	Water	500 ml glass (lab bottle blank & field equipment blank)	If unpreserved, 48 hr	Cool to 4 °C
Turbidity	180.1	Water	100 ml poly	48 hours	Cool to 4 °C
TDS, TSS	160.1, 160.2	Water	1 Liter poly	7 days	Cool to 4 °C
Ethanol	EPA 8015B	Water	3-VOA	14 days	pH <2, HCl Cool to 4 °C
Cations (Ca, Mg, Na, K)	EPA 6010	Water	500 Milliliter poly bottle	180 days	Nitric Acid pH<2 Cool to 4 °C
Major Ions – Chloride, Nitrate-N, Sulfate	EPA 300.1	Water	500 Milliliter poly bottle	28 days/48 hours	Cool to 4 °C
Total Organic Carbon	SM5310B	Water	2 VOA glass vials	28 days	HCl pH<2 Cool to 4 °C
Total Alkalinity	EPA 310.1/SM 2320B	Water	200 Milliliter poly bottle	14 days	Cool to 4 °C
Hardness	SM2340B/EPA 200.8	Water	100 milliliter Poly/Glass	6 months	pH <2, HNO ₃

Notes:

a = Metals include aluminum, antimony, arsenic, barium, beryllium, boron, cadmium, calcium, chromium, cobalt, copper, iron, lead, magnesium, manganese, molybdenum, nickel, potassium, selenium, silver, sodium, thallium, vanadium, zinc.

< Less than

°C Degrees Celsius

EPA U.S. Environmental Protection Agency

Poly Polyethylene

SW-846 Test Methods for Evaluating Solid Waste/Chemical Methods (EPA 1997)

ASTM American Society for Testing and Materials

SM Standard Methods for Examination of Water and Wastewater (1983)

VOA volatile organic analysis

ASAP As soon as possible

HCl Hydrochloric acid

12.0 SAMPLE HANDLING AND CUSTODY

General sample identification, labeling, handling and COC requirements are discussed in this section. COC procedures will be strictly adhered to during sample collection, transportation and laboratory handling to assure the identity of the samples. Improper sample and data handling and inadequate COC procedures affect the credibility and acceptability of analytical results, regardless of their accuracy or precision. COC documentation will document the processing of the sample from the time of collection to the time of analysis.

12.1 Sample Labeling and Handling

Sample containers with the necessary preservatives will be provided by the contract laboratories, UC Davis. After collection, samples will be labeled with all necessary information filled out using waterproof ink. At a minimum, each sample label will contain the following information:

- Project name;
- Site location;
- Sample identification code;
- Date and time of sample collection, with sampler's initials;
- Analyses required;
- Method of preservation, if used; and
- Sample matrix.

Each sample will be assigned a unique identification code (ID) according to sample location and date, as shown in Table 12-1. This ID will consist of the station code, followed by the date (4-digit year, 2-digit month, and the number for the day). For example, a sample collected at the Twin Peaks Adit on April 15, 2016 would be labeled TPA20160415.

Sample containers will be sealed in Ziploc® plastic bags and immediately placed on ice in an insulated cooler chilled to 4°C (degrees centigrade). Insulated coolers will be provided by the contract laboratories. Samples will be placed right-side up in a cooler with ice for delivery to the laboratory.

Table 12-1 Surface Water Sample Locations and Identifications

STATION CODE	LOCATION DESCRIPTION	LATITUDE AND LONGITUDE	EXAMPLE FIELD SAMPLING ID (STATION CODE, DATE - MMDDYY) (APRIL 15, 2016)
TPA	Twin Peaks Adit	38°39'44.37"N, 122°32'4.76"W	TPA20160415
BHA	Boiler House Adit	38°40'7.58"N, 122°32'20.64"W	BHA20160415
DTP	Drain Tunnel Portal	38°40'15.00"N, 122°32'10.41"W	DTP20160415
DKC	Kidd Creek 200 Feet downstream from Drain Tunnel	38°40'13.41"N, 122°32'8.44"W	DKC20160415
UKC	Kidd Creek upstream from Drain Tunnel	38°40'15.70"N, 122°32'10.95"W	UKC20160415
UKR	Kidd Creek upstream from Oat Hill Road	38°40'22.98"N, 122°32'25.65"W	UKR20160415
UBC	Bateman Creek upstream from Twin Peaks Mine	38°39'44.13"N, 122°31'59.93"W	UBK20160415
LBC	Bateman Creek downstream from Twin Peaks Mine	38°39'47.13"N, 122°31'56.72"W	LBC20160415
LRB	Bateman Creek lower reach	38°40'8.21"N, 122°31'44.58"W	LRB20160415
URB	Bateman Creek upper reach	38°39'27.91"N, 122°32'9.92"W	URB20160415
TPS	Twin Peaks seep into Bateman Creek	38°39'42.79"N, 122°32'0.20"W	TPS20160415
AZC	Azalea Flat Creek at mouth	38°40'30.97"N, 122°32'59.17"W	AZC20160415
CCU	Corona Creek Upstream from Mine	38°40'7.05"N, 122°32'20.96"W	CCU20160415
CFC	Corona Flat Creek above Kidd Creek	38°40'19.71"N, 122°32'42.15"W	CFC20160415
JCD	James Creek 1 km downstream	38°40'10.46"N, 122°31'3.71"W	JCD20160415
MNC	McNabb Creek at mouth	38°40'21.96"N, 122°32'46.15"W	MNC20160415
HCT	Hunters Camp water tank	TBD	HCT20160415
TPI	Twin Peaks Adit infiltration trench	TBD	TPI20160415
BHI	Boiler House Adit infiltration trench	TBD	BHI20160415
DTS	Drain Tunnel Portal settling basin (following construction)	TBD	DTS20160415
BHS	Boiler House Adit settling basin (following construction)	TBD	BHS20160415
TPS	Twin Peaks Adit settling basin (following construction)	TBD	TPS20160415
TPA	<i>Duplicate or Replicate Sample on TPA sample</i>	<i>Same as TPA</i>	<i>TPAD20160415</i>
TPA	<i>Matrix Spike on TPA sample</i>	<i>Same as TPA</i>	<i>TPAMS20160415</i>
FTB	<i>Filter Blank</i>	<i>Before first sample</i>	<i>FTB20160415</i>

12.2 Chain-of-Custody Procedures

The field team responsible for the collection of samples will sign and retain a copy of the COC form, document the method of shipment, and send the original with the samples. The original signed COC form (unless an equivalent laboratory field notebook page is used) will be sealed in a watertight plastic envelope and attached to the inside lid of the cooler. Coolers will be secured with strapping tape and a container custody seal applied that is over-strapped with clear strapping tape. All sample shipment coolers will be transferred from the field directly to the analytical laboratory. In cases where direct delivery to the analytical laboratory is not possible, samples will be stored in a refrigerator at no more than 4°C, but above freezing. Access to the refrigerator will be restricted to the field technician or coordinators and technicians.

A COC form (or equivalent laboratory notebook page) will be shipped or accompany the cooler to confirm transfer; the carrier waybill number will be recorded on the original COC. Commercial carriers are not required to sign the COCs. Copies of the COCs, notebook pages, and waybills will be forwarded to the monitoring program records by the field technician or coordinator.

Sample shipment will be scheduled to prevent exceeding any required holding times. Failure to conduct analyses within the required holding times may potentially require the qualification of associated analytical results and will prompt appropriate corrective and preventive action measures.

12.3 Laboratory Operations

Laboratory analyses will be managed in accordance with the laboratories' approved QA plans or manuals and the minimum requirements described in this section of the QAPP. In the event conflicts may exist between the QAPP and any of the laboratory QA plans, the more stringent requirement will apply.

12.3.1 Sample Receipt

When samples arrive at each laboratory, the designated laboratory custodian receiving the sample cooler will inspect the cooler custody seal. The custodian will sign the shipping COC (when utilized) and attach the carrier billing. The shipping COC and waybill will be archived in the laboratory's project file and a copy of the shipping COC and waybill will be forwarded for filing in the monitoring program records. The laboratory custodian will then open the cooler to inspect the samples for integrity and compare the number of containers and label information with the COC form attached to the inside of the cooler lid. Cooler temperatures will be checked and documented on the COC form. Broken custody seals, damaged sample containers, sample labeling discrepancies between container labels and the COC form, and analytical request discrepancies will be noted on the COC form. The QA Officer or field technician or coordinator will be notified of any such problems for their appropriate action.

Once any discrepancies are resolved, the laboratory custodian will enter the samples into an analytical custody log and will assign each sample a unique identification number that is cross referenced to the sample number assigned in the field. The identification number will be used by

the laboratory in its internal tracking system and the status of any given sample can be checked at any time by referring to the laboratory numbers on the COC form and in the laboratory logbooks. The laboratory custodian will then sign the COC form. The original COC will be routed to the laboratory's data management group. Copies of the COC forms documenting custody changes and documentation will be received and kept in the laboratory's project files. The original COC forms will remain with the samples until final disposition of the samples by the laboratory. Samples, extracts, or digestates will not be sent to another laboratory without the written authorization of the QA Officer. After sample disposal, a copy of the original COC will be sent to the QA Officer to be filed in the monitoring program records.

The following identifying information will be entered into the laboratory's database or logged into the laboratory's bound sample receiving logbook:

- Date and time of receipt
- Laboratory project number or work order number
- Project name and number
- Sample numbers, matrices
- Analyses required

12.3.2 Sample Storage and Security

Samples will be stored in secure, designated refrigerated areas as required for the analysis to be performed. A logbook or form will be maintained for each refrigerated area, and the temperature will be recorded each working day. At a minimum, the following procedures will be applied:

- Samples and extracts will be stored in a secure area controlled by the laboratory's designated sample custodian;
- Samples will be removed from the shipping container and stored in their original containers unless damaged; damaged samples will be disposed in an appropriate manner after notifying the lab manager and authorization to dispose is received and documented;
- Whenever samples are removed from storage, removal will be documented;
- Sample transfers will be documented on internal COC records;
- Samples and extracts will be stored after completion of analyses in accordance with contractual requirements, or until instructed otherwise by the lab manager; and
- Samples will not be stored with standards or sample extracts.

12.3.3 Sample Tracking

Laboratory personnel will use COC records, notebook pages, and databases to generate backlist reports of analyses for each sample. The reports will include the collection times along with the laboratory sample number, and will include a reference to the project title, field sample identifications, and sample matrix. Sample analyses will be scheduled on the basis of holding time considerations. Analytical assignments will be reviewed on a daily basis to ensure that holding times are not exceeded. If holding times are exceeded during laboratory custody, the

QA Officer or field technician or coordinator will be immediately notified for their appropriate action.

12.3.4 Sample Custody Records

Minimum requirements for laboratory sample COC controls are as follows:

- Samples will be stored in a secured area;
- Access to the laboratory will be through a monitored area; other outside access doors to the laboratory will be kept locked in accordance with local fire requirements;
- A visitor's log will be maintained, and visitors will be escorted while in the laboratory;
- Refrigerators, freezers, and other sample storage areas will be securely locked or maintained in a secured area;
- Only authorized personnel will have keys to locked sample storage area(s);
- Samples will remain in secure sample storage until removed for preparation or analysis;
- Sample transfers into and out of storage will be documented; and
- Custody records will be maintained by the laboratory's sample management group.

Samples, extracts, and digestates will be retained at the laboratory for at least 60 days after the laboratory's final analytical data report has been submitted, so that any potential analytical problems can be properly addressed. The samples, extracts, and digestates may then be discarded in an approved and environmentally safe manner unless otherwise directed by the lab manager.

13.0 ANALYTICAL METHODS

This project will focus on total mercury, aluminum, chromium, iron, manganese, and nickel. A larger list of metals will also be analyzed on a less frequent basis. In addition, field parameters (flow, dissolved oxygen, ORP, pH, temperature, specific conductance), alkalinity, turbidity, TSS, TDS, major ions (such as sulfate, chloride, nitrate), total organic carbon, and hardness will be monitored. Ethanol will be used to stimulate growth of sulfate-reducing bacteria within the mine. If reducing conditions are established within the mine, the metals will be removed by sulfate reduction and sulfide precipitation. If reducing conditions are not established using bacteria, then the pH will be lowered using sodium hydroxide. Therefore, ethanol (or TOC) and pH will be measured in the drain tunnel discharge to evaluate the effectiveness of the in-situ treatment. Tables 13-1 provide a list and description of the analytical methods used for this project for water samples. Additional analytical method information is provided in Chapter 7, Quality Objectives and Criteria for Measurement Data.

Sampling and analytical methods for fish and aquatic insects are provided in Appendix A.

Table 13-1 Summary of Analytical Parameters and Methods

PARAMETERS	MATRIX	METHOD REFERENCES	METHOD TYPE	METHOD DESCRIPTION
Total and Dissolved Metals ^a	Water	EPA 200.8 or 3010A / SW-846 6020A(ICP/MS)	Laboratory	Inductively coupled plasma mass spectrometry (ICP/MS)
Total Mercury	Water	EPA Method 1631E/1669	Laboratory/Field Sampling	Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry (CVAFS)/sampling Ambient Water for Trace Metals at EPA Water Quality Criteria Levels
Total Methyl- Mercury	Water	EPA Method 1630/1669	Laboratory/Field Sampling	Methyl Mercury in Water by Distillation, Aqueous Ethylation, Purge and Trap, and CVAFS/Sampling Ambient Water for Trace Metals at EPA Water Quality Criteria Levels
Major Anions – Chloride, Sulfate, Nitrate-N	Water	EPA 300.1	Laboratory	Determination of Inorganic Anions in Drinking Water by Ion Chromatography
Total Alkalinity as CaCO ₃	Water	EPA 310.1/SM 2320B	Laboratory	Alkalinity, Titrimetric, pH 4.5
Hardness (Ca, K, Mg, Na)	Water	SM2340B/EPA 200.8	Laboratory	Hardness by calculation, and ICP/MS
Total Organic Carbon	Water	SM5310B	Laboratory	Total Organic Carbon
Turbidity	Water	EPA 180.1	Laboratory	Turbidity by Nephelometry
Ethanol	Water	EPA 8015B	Laboratory	GC Method modified
Temperature	Water	SM2550	Field	Temperature
pH	Water	EPA 150.1	Field	pH
Dissolved Oxygen	Water	EPA 360.1	Field	Dissolved Oxygen
ORP	Water	SM2580 AB	Field	Oxidation-Reduction Potential (ORP)
Specific Conductance	Water	EPA 120.1	Field	Specific Conductance (SC)
Tracer (sulforhodamine B)	Water	See instrument manual	Field	Fluorimeter (data logger)
Total and Dissolved Metals ^a	Water	EPA 200.8 or 3010A / SW-846 6020A(ICP/MS)	Laboratory	Inductively coupled plasma mass spectrometry (ICP/MS)

Notes:

a = Metals include aluminum, antimony, arsenic, barium, beryllium, boron, cadmium, calcium, chromium, cobalt, copper, iron, lead, magnesium, manganese, mercury, molybdenum, nickel, potassium, selenium, silver, sodium, thallium, vanadium, zinc

EPA U.S. Environmental Protection Agency

SW-846 Test Methods for Evaluating Solid Waste/Chemical Methods (EPA 1997)

SM Standard Methods for the Examination of Water and Wastewater (1983)

ASTM American Society for Testing and Materials

14.0 QUALITY CONTROL

Quality assurance checks include laboratory and field methods. Laboratory quality assurance checks may include the use of blank, spiked, split and duplicate samples, calibration checks and internal standards. These samples will be handled and analyzed using the same procedures as the primary samples.

14.1 Field Quality Control Samples

Field QC samples should be collected in the field and used to evaluate the quality of the field sampling activities. The field QC samples are listed in the work plan and indicated in Section 10 of this QAPP. Each project-specific work plan will address the specific field QC sampling requirements in the associated QAPP. Field QC samples may consist of field duplicate samples, field method blanks and/or field equipment rinsate blanks.

14.1.1 Field Duplicate Samples

Field duplicate samples may be collected to assess the accuracy, precision and overall quality of the sampling and analytical procedures. Field duplicate samples are collected at the same time and from the same source as the original sample. Duplicate samples will be collected, numbered, packaged, and sealed in the same manner as other samples, and submitted blind to the laboratory for identical analyses as the original sample.

14.1.2 Field Method Blank Samples

Field method blanks consist of source water (often commercially distilled water or laboratory-prepared deionized water, depending on the specific QC requirements) that is placed into the sampling containers at the same time and location of a field sample. The field blank sample is handled in the same manner as other samples, and submitted blind to the laboratory for identical analyses as the other samples. Field blanks are used to evaluate any contamination present during sampling and/or laboratory contamination.

14.1.3 Field Equipment Rinsate Blank Samples

Field equipment rinsate blanks consist of source that is placed into the sampling containers at the same time and location as a field sample. The equipment rinsate blank sample is collected in the same manner as other samples, utilizing any non-dedicated, decontaminated sampling equipment (sample tubing, filters, shovels, etc.). These samples are submitted blind to the laboratory for identical analyses as the other samples. Field equipment rinsate blanks are used to evaluate decontamination procedures of sampling equipment.

14.2 Laboratory Quality Control Samples

Laboratory QC checks are designed to determine analytical precision and accuracy, demonstrate the absence of interferences and contamination from glassware and reagents, and ensure comparability of data. Laboratory QC checks consist of LCSs, method blank samples, MS/MSD samples, laboratory duplicate samples, and other checks specified in the methods. The laboratory

also will complete initial calibrations and continuing calibration checks according to specified analytical methods.

14.2.1 Method Blanks

Method blanks will be used for the laboratory processes, as defined by the governing method. A method blank is a volume of deionized water that is carried through the entire sample preparation and analysis procedure. The method blank volume or weight will be approximately equal to the sample volumes or sample weights being processed. Method blanks are used to monitor interference caused by constituents in solvents and reagents and on glassware and other sampling equipment. A method blank is prepared and analyzed with each analytical batch of 20 or fewer samples prepared.

14.2.2 Matrix Spikes

A spike is a sample to which is added a known amount of analyte(s) before analysis. From the concentrations of the analyte in the spiked and unspiked samples, a percent recovery is calculated. Many samples show matrix effects in which other sample components interfere with the determination of the analyte. The value of the percent recovery indicates the extent of the interference. A matrix spike is prepared by adding an analyte to a subsample of a field sample before sample preparation and analysis. For multi-analyte methods, a representative suite containing all of the analytes is used in the matrix spike. If matrix spikes are spiked at too low of a concentration (i.e., less than 3-5 times the native concentration), they will be repeated at a higher spike concentration. An analytical spike is prepared by adding analyte to an aliquot of a processed sample prior to analysis, and is used to determine whether the analysis system provides results that are representative of the sample when a matrix spike is outside its limits.

14.2.3 Laboratory Control Samples (Verification Solutions)

A LCS, or a blank spike, is an aqueous or solid control sample of known composition that is analyzed using the same sample preparation, reagents, and analytical methods employed for the monitoring program samples. An LCS is obtained from an outside source or is prepared in the laboratory by spiking reagent water or a clean solid matrix for a stock solution that is different than that used for the calibration standards. The LCS is the primary indicator of process control used to demonstrate whether the sample preparation and analytical steps are in control, apart from sample matrix effects. LCSs contain the target analytes identified in the method.

14.2.4 Laboratory Duplicate Samples

Duplicate samples are samples that have been divided into two portions at some step in the measurement process. Each portion is then carried through the remaining steps of the process. Duplicate samples provide information on the precision of the operations involved. Analytical duplicates are a pair of subsamples from a field sample that are taken through the entire preparation and analysis procedure; difference between the results indicates the precision of the entire method in the given matrix. Under the laboratory protocols, the matrix spike is duplicated to provide a matrix spike duplicate. Matrix spike duplicates will be prepared for every analytical batch of at least 20 samples. Analytical duplicates are prepared by taking two aliquots of a process sample and analyzing them in the same manner. Both matrix and analytical spike duplicates are used to monitor the precision of the analytical process.

For water samples, the laboratory will analyze one laboratory duplicate sample per sample delivery group. A sample delivery group is defined as a group of up to 20 samples received within a 14-day period.

Laboratory internal QC checks are summarized in Table 14-1. Table 14-1 also indicates the required frequency, acceptance criteria, and corrective action for each QC check. Each of these checks and their frequencies are discussed below.

Table 14-1. Summary of Laboratory Quality Control Samples

QC CHECK SAMPLE	FREQUENCY	ACCEPTANCE CRITERIA	CORRECTIVE ACTION
Laboratory control samples	5 percent or 1 per batch, whichever is more frequent	Accuracy 85 to 115% (varies based on method) RPD 15% to 25% (varies with media and method)	<ol style="list-style-type: none"> 1. Evaluate other QC samples in batch. 2. Correct the problem. 3. Repeat the analysis. 4. Flag data in report.
Method blank	5 percent or 1 per batch, whichever is more frequent	Less than the PQL	<ol style="list-style-type: none"> 1. Terminate the analysis. 2. Correct the problem. 3. Reanalyze affected samples. 4. Flag data in report.
Instrument blank	At the beginning, end, and after every 10 samples during analysis sequence	Less than the PQL	<ol style="list-style-type: none"> 1. Terminate the analysis. 2. Correct the problem. 3. Recalibrate and reanalyze samples. 4. Flag data in report.
Laboratory duplicate sample (analyze duplicate aliquots from an actual field sample submitted for this project)	5 percent or 1 per batch, whichever is more frequent	RPD of 20 (varies based on method)	<ol style="list-style-type: none"> 1. Evaluate other QC samples in the batch. 2. Flag the data in report.
Matrix Spikes and Duplicate Matrix Spikes	5 percent or 1 per batch, whichever is more frequent	Accuracy 75 to 125%	<ol style="list-style-type: none"> 1. Evaluate other QC samples in the batch. 2. Flag the data in report.
Matrix Duplicate	5 percent or 1 per batch, whichever is more frequent	RPD of 25	<ol style="list-style-type: none"> 1. Evaluate other QC samples in the batch. 2. Flag the data in report.

Notes:

- %R Percent recovery
- RPD Relative percent difference
- PQL Practical quantitation limit
- QC Quality control

15.0 INSTRUMENT/EQUIPMENT TESTING, INSPECTION, AND MAINTENANCE

15.1 Calibration of Sampling Equipment

For water sampling, instrumentation and equipment used will generally be limited to filtration apparatus and field meters. The filtration apparatus will be used and maintained in accordance with the manufacturer's instructions. Field meters, including the air monitoring analyzer, will be calibrated following the manufacturer's instructions for each analyte of interest and at least once per sampling event.

15.2 Calibration of Laboratory Equipment

All analytical measurement instruments and equipment used by the laboratories will be controlled by a formal calibration and preventive maintenance program. At a minimum, each laboratory will require that equipment be of the proper type, range, accuracy, and precision to provide data compatible with specified requirements. All instruments and equipment that measure a quantity, or whose performance is expected at a stated level, are subject to calibration. In addition, each laboratory's preventive maintenance program will include the following, as a minimum:

- A listing of the instruments and equipment that will be used;
- The frequency of maintenance considering manufacturer's recommendations and previous experience with the equipment; and
- A file for each instrument containing a list of spare parts maintained, external contracts, and a listing of the items to be checked or serviced during maintenance.

16.0 INSTRUMENT/EQUIPMENT CALIBRATION AND FREQUENCY

16.1 Calibration Methods

Physical and chemical calibrations will be performed within each laboratory as described in this subsection. Physical calibration refers to physical measurements that are made on equipment to verify or provide corrections to the observed data. Physical calibrations will be documented on data sheets that are designed for each specific application. At a minimum, the information recorded will include the date, analyst, instrument identification, identification of reference standard, expected values, measured values, and correction factors, if applicable.

Chemical calibration or standardization refers to operations in which instrument response is related to analyzed concentration. The minimum requirements for chemical calibration will be as specified in the applicable method. Chemical calibrations consist of initial and continuing calibrations, which are documented in several ways depending on the type of instrument. For non-computerized data systems such as strip chart recorders and meter readouts, the instrument responses will be transcribed, along with other pertinent information, onto data sheets for each specific analysis. When computerized data systems are used, the data will be collected and stored in computer files, as well as hard-copy printouts, which may either be included with the data package or kept in a central record. With computerized data systems, the run logs provide a cross-reference to the calibration runs. At a minimum, the information recorded for the calibrations will include the data, analyst, instrument identification, standard identification and concentrations, raw instrument responses, file descriptor, and calibration parameters such as regression coefficients, correlation coefficients, or response and calibration factors.

Initial calibration consists of the establishment of a calibration or standard curve, which associates instrument response and analyzed concentration. The curve is constructed by measuring the responses of a series of standard solutions containing the analytes of interest at known concentrations. This initial calibration will be verified each working day by measurement of one or more calibration standards.

16.2 Calibration Apparatus

The use of calibration apparatus, including field instruments and data loggers, will be according to the manufacturer's instructions or the laboratory's SOPs.

16.3 Calibration Standards

Primary standards will be obtained as either neat materials, which will be used to prepare stock standard solutions, or as prepared solutions to be used as stock standards. Records will be maintained on primary standards that include date of receipt, source, purity, composition, storage

conditions, and expiration dates. Primary standards will be traceable to National Institute of Standards and Technology standards, or will be vendor-certified. The preparation of stock, intermediate, and working standard solutions will be documented in standards preparation logbooks. Each stock, intermediate, and working standard will be assigned a number to permit traceability of preparation from stock to working standards and to reference the analysis of the standards. Logbooks will be completed by the appropriate analysts as they prepare standards and will be subject to supervisory review. At a minimum, working standards will be labeled with preparation data, and the number or designation of the logbook where information on the standard is recorded. Measurements made during standards preparation will also be recorded.

16.3 Calibration Frequency

The frequency of instrument calibration will be according to the manufacturer's instructions or the laboratories' SOPs. Table 16-1 includes a summary of calibration requirements.

Table 16-1. Summary of Laboratory and Field Calibration Requirements for Major Parameters

PARAMETER	METHOD REFERENCE	CALIBRATION STANDARD AND FREQUENCY	ACCEPTANCE CRITERIA	CORRECTIVE ACTION
Total Metals	EPA 200.8/SW-846 6020A	Mass spectrometer tuning solution representing all of the mass regions of interest analyzed to verify that the resolution and mass calibration of the instrument are within required specifications.	Mass calibration must be no more than 0.1 atomic mass units (amu) from the true value. Peak resolution must also be verified to be less than 0.9 amu full width at 10 percent peak height. Four integrations (measurements) of tuning solution values must be within a relative standard deviation of 5 percent for the analytes contained in the tuning solution.	Mass calibration must be adjusted to the correct value.
		Low-level initial calibration (LLICV) standard analyzed daily and at the end of each analysis batch.	All analytes measured values are within ± 30 percent of the expected value.	Terminate the sequence, correct the problem, recalibrate the instrument, and reanalyze.
		Initial calibration verification (ICV) immediately following daily calibration (using a second source standard).	Measured value within ± 10 percent of the expected value.	Terminate the analysis, correct the problem, and recalibrate the instrument
		Initial and Continuing Calibration blank (ICB and CCB) immediately following the daily calibration, after every 10th sample, and at the end of the analytical run.	Below the detection limit.	Terminate the analysis, correct the problem, recalibrate the instrument, and reanalyze the previous 10 samples
		Continuing calibration verification (CCV) immediately following the daily calibration, after every tenth sample, and at the end of the analytical run	Measured value within 10 percent of the expected value.	Terminate the analysis, correct the problem, recalibrate the instrument, and reanalyze the previous samples following the last acceptable CCV and CCB.
		Interference check sample (ICS) at the beginning of each analytical run or weekly after five consecutive daily analyses are within 20% of the expected value	Measured value within 20 percent of the expected value.	Terminate the analysis; determine and correct the cause of the change, update the interelement correction factor, and recalibrate the instrument

Table 16-1. Summary of Laboratory and Field Calibration Requirements for Major Parameters

PARAMETER	METHOD REFERENCE	CALIBRATION STANDARD AND FREQUENCY	ACCEPTANCE CRITERIA	CORRECTIVE ACTION
Total Mercury	EPA Method 1631 E	Initial Blanks (3 System blanks or 3 Bubbler blanks)	Bubbler blanks must contain <50 pg Hg. System blanks must contain <0.50 ng/L Hg.	Terminate the analysis, correct the problem, reanalyze samples.
		Five non-zero calibration standards. Lowest calibration point must be at the Minimum Level (ML) of 0.5 ng/L	Relative Standard Deviation (RSD) of calibration factors (CF) for all points must be ≤ 15 %	If RSD ≤ 15%, calculate the recovery for the lowest standard using mean calibration factor (CF _m). If the RSD ≤ 15% and the recovery of the lowest standard is in the range of 75-125%, the calibration is acceptable and CF _m may be used to calculate the concentration of Hg in samples. If RSD > 15% or if the recovery of the lowest standard is not in the range of 75-125%, recalibrate the analytical system and repeat the test.
		Initial Precision and Recovery (IPR)	Recovery must be within 79 -121 % RSD must be within 21%	Analyze four replicates of IPR solution. If outside of the acceptance range, correct problem, and repeat the test.
		Method blanks analyzed after every three or four samples.	Bubbler blanks must contain <50 pg Hg. System blanks must contain <0.50 ng/L Hg.	Terminate the analysis, correct the problem, reanalyze samples.
		On-going precision and recovery (OPR) at beginning and end of each batch.	Recovery must be within 77 to 123 %	If system is not in control, correct the problem, repeat ongoing precision and recovery test. If system performance is verified at the end of the sequence using the OPR, analysis of samples and blanks may proceed without recalibration, unless more than 12 hours has elapsed since verification of system performance.

Table 16-1. Summary of Laboratory and Field Calibration Requirements for Major Parameters

PARAMETER	METHOD REFERENCE	CALIBRATION STANDARD AND FREQUENCY	ACCEPTANCE CRITERIA	CORRECTIVE ACTION
Methyl Mercury	EPA Method 1630	Three method blanks (distillation blanks) with each analytical batch.	Mean blank value < 0.045 ng/L methyl mercury. Variability should be <0.015 ng/L methyl mercury.	Indicates problem with system and unacceptable for low level ambient analysis. Investigate and repeat analysis.
		One Ethylation Blank after OPR and prior to samples. Used to blank correct standards.	Value must be <2 pg methyl mercury.	Indicates problem with reagent water or solutions. Investigate, make new batch of ethylation blank solution, repeat analysis.
		Five non-zero calibration standards. Lowest calibration point must be at the ML of 0.06 ng/L methyl mercury.	RSD of CF for all points must be ≤ 15 %	If RSD ≤ 15%, calculate the recovery for the lowest standard using CFm. If the RSD ≤ 15% and the recovery of the lowest standard is in the range of 75-125%, the calibration is acceptable and CFm may be used to calculate the concentration of Hg in samples. If RSD > 15% or if the recovery of the lowest standard is not in the range of 75-125%, recalibrate the analytical system and repeat the test.
		IPR	Recovery must be within 69 -131 % RSD must be within 31 %	Analyze four replicates of IPR solution. If outside of the acceptance range, correct problem, and repeat the test.
		Method blanks analyzed after every three or four samples.	Bubbler blanks must contain <50 pg Hg. System blanks must contain <0.50 ng/L Hg.	Terminate the analysis, correct the problem, reanalyze samples.
		OPR at beginning and end of each batch (within 12-hours).	Recovery must be within 67 to 133 %	If system is not in control, correct the problem, repeat ongoing precision and recovery test.

Table 16-1. Summary of Laboratory and Field Calibration Requirements for Major Parameters

PARAMETER	METHOD REFERENCE	CALIBRATION STANDARD AND FREQUENCY	ACCEPTANCE CRITERIA	CORRECTIVE ACTION
				If system performance is verified at the end of the sequence using the OPR, analysis of samples and blanks may proceed without recalibration, unless more than 12 hours has elapsed since verification of system performance.
Total Organic Carbon	EPA Method 415.3	Spectrophotometer Performance Check standard is analyzed to demonstrate performance of spectrophotometer.	Reading must be within 10 percent of the expected absorbance value.	Terminate the analysis, correct the problem, and recalibrate the instrument.
		Initial five point calibration verification with low standard defining the minimum reporting limit (RL).	Measured value within ± 10 percent of the expected value.	Terminate the analysis, correct the problem, and recalibrate the instrument.
		CCV after every tenth sample, and at the end of the analytical run.	Measured value within 15 percent of the expected value.	Terminate the analysis, correct the problem, recalibrate the instrument, and reanalyze the previous samples following the last acceptable CCV.
		ICB and CCB immediately following the daily calibration, after every 10th sample, and at the end of the analytical run.	Below the detection limit.	Terminate the analysis, correct the problem, recalibrate the instrument, and reanalyze the previous 10 samples.
Major Anions – Chloride, Sulfate, Nitrate-N	EPA 300.1	Initial Calibration Standards, 5 calibration standards for each analyte (one at lower and one at upper range of each analyte).	Within 85 to 115 percent accuracy.	Terminate the analysis, correct the problem, and recalibrate the instrument.
		Initial Calibration Check Standard, daily	Within 85 to 115 percent.	Terminate the analysis, correct the problem, and recalibrate the instrument.
		Continuing Calibration Check Standard, after every 10 samples.	Within 85 to 115 percent.	Terminate the analysis, correct the problem, recalibrate the instrument, and reanalyze the previous 10 samples.
		End Calibration Check Standard, after last field sample.	Within 85 to 115 percent.	Terminate the analysis, correct the problem, recalibrate the instrument, and reanalyze the previous samples after last calibration standard within acceptance criteria.

Table 16-1. Summary of Laboratory and Field Calibration Requirements for Major Parameters

PARAMETER	METHOD REFERENCE	CALIBRATION STANDARD AND FREQUENCY	ACCEPTANCE CRITERIA	CORRECTIVE ACTION
		Laboratory Reagent blank, with each batch of samples.	Below the detection limit.	Terminate the analysis, correct the problem, and reanalyze the samples.
Total Alkalinity	EPA 310.1/SM 2320B	Standardize pH meter using standard buffer solutions, or electrically operated titrator according to manufacturer's instructions, daily. Calculate normality of standard acids (0.1N or 0.02N) by potentiometric titration with Na ₂ CO ₃ .	Expected amount of Na ₂ CO ₃ for normality of standard acid. pH readings within 0.05 pH units.	Repeat titration. Make fresh reagents.
Turbidity	EPA 180.1	Measure standards on the turbidimeter covering range of interest.	At least one standard should be run in each instrument range to be used. Turbidities exceeding 40 units, dilute with turbidity-free water.	Recalibrate the instrument.
TSS, TDS	EPA 160.1, 160.2	Gravimetric. Drying oven, 180° C.	Oven temperature should be verified to within ± 2° C. Analytical balance calibrated and capable of weighing to 0.1 mg	Recalibrate the instrument.
Hardness	SM2340/EPA 200.8	Calculation method, sum of calcium and magnesium concentrations, expressed as calcium carbonate.	See metals	See metals
Ethanol	EPA 8015B	Initial Calibration	Within 85 to 115 percent	Terminate the analysis, correct the problem, and recalibrate the instrument
		Initial five point calibration verification with low standard defining the minimum reporting limit (RL).	Measured value within ± 10 percent of the expected value.	Terminate the analysis, correct the problem, and recalibrate the instrument.
		Continuing Calibration	Within 85 to 115 percent	Terminate the analysis, correct the problem, and recalibrate the instrument
pH (field)	EPA 150.1	Calibrate using 3 pH buffer standard solutions, daily.	Within 0.05 pH unit	Rinse electrode, repeat. Replace with fresh buffer solutions.
ORP (field)	SM2580 AB	Follow manufacturer's instructions for calibration.	Follow manufacturer's instructions.	Follow manufacturer's instructions.

Table 16-1. Summary of Laboratory and Field Calibration Requirements for Major Parameters

PARAMETER	METHOD REFERENCE	CALIBRATION STANDARD AND FREQUENCY	ACCEPTANCE CRITERIA	CORRECTIVE ACTION
Dissolved Oxygen (field)	EPA 360.1	Follow manufacturer’s instructions.	Follow manufacturer’s instructions.	Follow manufacturer’s instructions.
Conductivity (SC) (field)	EPA 120.1	Measure 0.01 M KCl solution, daily. Reading at 25 C is 1413 µmho/cm, adjusted for temperature.	Within 10 µmho	Correct for temperature. Repeat. Replace KCl solution.
Tracer (sulforhodamine B)	Fluorimetry	Follow manufacturer’s instructions.	Follow manufacturer’s instructions	Follow manufacturer’s instructions

17.0 INSPECTION/ACCEPTANCE OF SUPPLIES AND CONSUMABLES

All purchased supplies and consumables that support field monitoring and sampling activities or that have a direct relationship to sample quality (e.g. sample containers, decontamination supplies, distilled/de-ionized water) will be inspected upon receipt. At a minimum this inspection will check:

- Part number/physical description matches requisition;
- Supplies are intact and undamaged;
- All required components/documentation is included; and,
- Any non-conforming items will be documented and returned to the supplier for replacement, re-work or other action as necessary.

18.0 NON-DIRECT MEASUREMENTS (EXISTING DATA)

The existing data that will be utilized for this project were compiled from various sources to characterize: mine waste at Corona and Twin Peaks Mine; mine drainage from Corona Mine portal and Twin Peaks portal (1997, and 1999 through 2001, and 2007); surface water from Kidd Creek and Bateman Creek (1997, 1999 through 2001); sediment from Bateman Creek.

Groundwater from the area of the mines is not used as a source for drinking water by humans. Groundwater from the mines discharges either as drainage from mine openings, or directly to surface water within Kidd and Bateman creeks within a short distance of each site.

A summary of the existing data and the data needs is presented in the document titled, *Data Quality Objectives for the Corona and Twin Peaks Mines*, August 2012, prepared for Tuleyome by Burleson (Burleson, 2012a). Site characterization work was conducted in 2012 by Tuleyome under Grant E1183005 and involved collecting air, mine waste, soil nutrient, biota, and surface water from Kidd Creek, Bateman Creek, other onsite seeps and creeks, and adit drainages. Surface water quality data was summarized in a memorandum (UC Davis and McCord Environmental, 2013). Fish and biota data was summarized in the data report, *Corona/Twin Peaks Mine Remediation Project: 2012 Baseline Stream Biota Mercury Monitoring* (Slotton, 2013).

19.0 DATA MANAGEMENT

19.1 Field Data Verification and Reporting

Field data will be recorded in field logbooks as the measurements are taken. Integrity of daily field instrument calibrations will be performed by the field staff and/or the Field Manager according to the instrument calibration procedures provided by the equipment manufacturer. The field instrument QC measurements will be reviewed to assure the accuracy and precision of the field screening measurements. As a standard QC procedure, the field staff and/or field coordinator will conduct verification of field data calculations and documentation entries. Prior to entry into the database, field data will be screened to ensure that no transcription errors occurred.

19.2 Laboratory Data Verification/Validation

Any anomalies or limitations for the use of the data will be documented as part of the laboratory's analytical data packages. The laboratories will establish calibration curves and calculation of sample concentrations from instrument responses. Raw laboratory data will be converted to sample concentrations using formulas defined by applicable laboratory SOPs. The calculations will be performed by computerized data systems interfaced to the instruments by personnel calculators, or through programs installed on stand-alone personnel computers. Each laboratory analyst will be responsible for the reduction of the raw data that they generate. At a minimum, such activities will include:

- Reduction of raw data generated to reportable values;
- An initial review of analytical and QC data;
- Performance of manual calculations and transfer of data onto forms, laboratory reports, and laboratory databases;
- Preparation of computer files for instrumental calculations;
- Generation of data forms for the analytical reports;
- Copying of relevant forms and logs for inclusion in the laboratory reports;
- Submittal of the laboratory report to a supervisor for a QA/QC review; and
- Resolution of discrepancies noted during the QA/QC review.

For non-instrument methods and for methods using instruments without computerized data systems that require manual calculations, the responsible analyst will enter bench-generated data into bound laboratory workbooks with form-specific instrument responses, standard and spike concentrations, sample numbers, and other pertinent information. For instruments that are directly coupled to computerized data systems, raw data consist of instrument responses in the form of printer output or computer-generated data files. Printer output will be filed by sample batch, and the data files archived on disk or magnetic tape. Computer data files will be identified

by unique, sequential descriptors cross-referenced in the run logs to the analysis sequence. At a minimum, strip chart recordings will be labeled with the following information:

- Sample identification number;
- Date and time of analysis;
- Instrument identification;
- Name of analyst(s);
- Applicable operational parameters;
- Date file identification; and
- Positively identified elements or compounds.

Completed analytical packages (hard copies and EDDs) for each sampling round will be routed to the QA Officer for evaluation and preparation of reports. All data will be screened to ensure that data packages are complete and do not contain any obvious entry errors; any observed problems will be referred to the affected laboratory for resolution. Data will be evaluated against the SWAMP program deliverable requirements. At a minimum, the screening process will verify:

- COC forms were properly maintained from the field, to the laboratory, and through all analytical procedures;
- Laboratory reports are complete and contain no transcription errors or omissions;
- Holding time requirements were met;
- Method blanks met method requirements;
- Quantitation limits did not exceed established values; and
- SWAMP format for chemistry EDD.

19.3 Data Storage and Retrieval

Data collected at the site must be in accordance with the QAPP and SWAMP, from sample collection in the field to data quality evaluation and data entry. Each entity collecting and analyzing data is responsible for its own data quality. Electronic analytical data must be formatted in accordance with SWAMP-compatible requirements.

Tuleyome and MEI will retain records of all monitoring information, including, all sampling logs and field notes, all calibration and maintenance records and all original strip chart recordings for continuous monitoring instrumentation, copies of all reports, a copy of the this QAPP for a period of at least five years from the date of the sample, measurement, report, or application. All records will be maintained as part of the monitoring program records. Receiving water data will be uploaded to CEDEN.

Group C Assessment and Oversight

20.0 ASSESSMENTS AND RESPONSE ACTIONS

20.1 Field and Laboratory Assessments

At the discretion of the QA Officer, on-site assessments of sampling practices or analytical laboratory activities may be performed periodically over the course of the monitoring program. The types of assessments include the following:

Readiness Review: Prior to the commencement of a scheduled phase of field-work, a readiness review may be conducted to determine if all components of the project are in place so that work can commence on a specific phase.

Project Surveillance: Continual or frequent monitoring of project status and analysis of project records may be conducted to ensure that the specific requirements of the QAPP and associated project planning documents are being fulfilled.

Technical Systems Audit: A thorough and systematic on-site qualitative audit of facilities, equipment, personnel, training, procedures and record keeping may be conducted for conformance to the QAPP.

Performance Evaluation: An audit of an analytical laboratory where performance evaluation samples are submitted and compared with routinely obtained samples. If required, the following information must be provided:

- The constituents to be measured;
- The target concentration ranges, the timing/schedule for performance evaluation sample analysis; and
- The aspect of measurement quality to be assessed (e.g. bias, precision, and detection limit).

Audit of Data Quality: An audit uses data verification methodology to examine how data were handled, what judgments were made, and whether any uncorrected mistakes were made. If an audit is planned, it should be performed prior to the production of a final report in order to correct any systematic data reduction errors. This step is in addition to standard minimum data verification.

20.2 Independent Technical Review

An independent technical review of all deliverable reports will be performed by the QA Officer or their designee and all comments resolved prior to submittal for external review. Any comments received from external review will be resolved and the revised reports re-reviewed

prior to resubmittal. Copies of all as-submitted reports and associated correspondence will be retained as monitoring program records. The following methodologies are options for review:

Peer Review: The review should assess the assumptions, calculations, extrapolations, alternative interpretations, methods, acceptance criteria and conclusions documented in the project report. The name, title, and position of any peer reviewer should be included and any comments appended to the final report.

Data Quality Assessment: Data quality assessment involves the use of statistical tools to determine if the data meet the assumptions that the Data Quality Objectives (DQO) and data collection design were developed under, and whether the total error in the data is tolerable. This assessment consists of five steps that relate the quality of the results to the intended use of the data:

- Step 1: Review DQOs and sampling design
- Step 2: Conduct preliminary data review
- Step 3: Select statistical test(s), as appropriate, to evaluate data quality
- Step 4: Verify assumptions
- Step 5: Draw conclusions about the quality of the data (data report will not include interpretation of results, but will state conclusions regarding the quality of the results).

If, as a result of the data assessment process, it is determined that data do not satisfy all DQOs, then corrective action(s) should be recommended. Corrective actions include, but are not limited to, revision of the DQOs, based on the results of the investigation, or collection of more information or data. It may be determined that corrective actions are not required, or the decision process may continue with the existing data, with recognition of the limitations of the data.

20.3 Documentation of Assessments

The field assessment and independent technical review will be documented, reported to management and retained as monitoring program records.

20.4 Corrective and Preventive Action

Corrective action and preventive action is required in response to a nonconformance. A nonconformance is defined as a potential or existing condition that may have an adverse impact on data quality. A nonconformance may be observed during routine project work or during any assessment process. Corrective and preventive action is applicable to both the field and laboratory procedures. In general, any member of the project team who identifies a nonconformance can initiate a corrective and preventive action.

The corrective and preventive action process is designed to identify, correct, and prevent or reduce the likelihood of recurrence of any nonconformance. The process consists of the following steps:

- Identify the problem constituting the potential nonconformance;
- Communicate the problem to the QA Officer, identifying the source of the quality requirement that has not been followed, applicable SOP, laboratory QA/QC procedure, applicable section of QAPP, as well as a description of the nonconformance;
- Project personnel assist in identifying the root cause of the nonconformance and identifying appropriate corrective actions as well as preventive actions;
- QA Officer and Project Manager approve proposed corrective and preventive actions and ensure that they are assigned to appropriate personnel with achievable deadlines;
- Assigned personnel (or external laboratory staff) implement actions and confirm completion;
- The QA Officer verifies completion and verifies that the actions taken were effective; and
- Any impact on data quality that was unable to be corrected must be noted in the data or relevant report.

The resolution of all quality issues will be documented in an internal memo or e-mail under the direction of the QA Officer and retained in the monitoring program records.

21.0 REPORTS TO MANAGEMENT

Table 21-1 lists the reports that will be written during the project. The analytical data collected under this QAPP will be provided in the Final Report and provided in a SWAMP-compatible electronic database format.

Table 21-1 QA Management Reports

TYPE OF REPORT	FREQUENCY	PROJECTED DELIVERY DATES	ENTITY RESPONSIBLE FOR REPORT PREPARATION	REPORT RECIPIENTS
Quarterly Progress Reports (includes other reports specified by Grant)	Quarterly	April 2016-September 2018	MEI	Tuleyome, DFW
Final Report (includes all monitoring data generated)	Once	November 2018	MEI, Burleson, Green, Smith, TKT	Tuleyome, DFW

Group D Data Validation and Usability

22.0 DATA REVIEW, VERIFICATION, AND VALIDATION REQUIREMENTS

22.1 Data Review and Verification Requirements

Data review is the laboratory in-house examination to ensure that the data have been processed correctly. Data verification refers to the routine checks that the field sampling coordinator or project QA officer, or laboratory staff, conduct in ensuring that the sampling plan and QAPP was followed, as applicable.

At a minimum, data verification will include evaluation of sampling documentation/representativeness, technical holding time, instrument calibration and tuning, field and lab blank sample analyses, method QC sample results, field duplicates and the presence of any elevated detection limits. The output of data verification includes the verified data package from the laboratory with any applicable laboratory qualifiers. The laboratory will provide analytical results in SWAMP-compatible electronic format. In addition, the lab will provide hard copies of the data with supporting laboratory quality assurance documentation.

22.2 Validation Methods

Data validation refers to the confirmation by independent examination of the data and provides objective evidence that the analytical method-specific and sample-specific procedures were followed by the laboratory and met the particular requirements for the intended use of data. Data validation methodology will differ according to the project's DQOs. At a minimum, the following items are addressed as part of a cursory data validation process:

- Chain-of-custody forms and laboratory data sheets will be checked to verify that appropriate analyses were run and that the samples were analyzed within specified holding times;
- Review of duplicate and blank samples will be used to evaluate method precision by the laboratory;
- An overall review of the sample delivery group will be conducted to evaluate the overall quality of the data. Included will be a review for potential transcription errors, detection limit discrepancies, data omissions and suspect or anomalous values; and
- Field data will be reviewed. Anomalous or suspect values will be noted and an explanation provided.

The analytical laboratory that generates the data will be responsible for in-house validation of the results. Laboratory validation checklists for inorganic analyses have been developed for implementation by the laboratory, as indicated in Table 22-1. The minimum output of data validation activities is a set of validated data with any additional qualifiers and a data validation report summarizing the findings of the data validation.

Table 22-1 Definitions of Data Flags and Qualifiers for Inorganic Data

TYPE	DESCRIPTION	VALUE
Laboratory Flag^a		
N	Laboratory spike sample results outside control limits	--
*	Laboratory duplicate results outside control limits	--
E	Sample results qualified because of interference (graphite furnace atomic absorption [GFAA] analytical spike or inductively coupled plasma [ICP] serial dilution	--
M	Duplicate injection precision for GFAA analysis outside control limits	--
W	Post-digestion spike for GFAA outside control limits	--
+	Correlation coefficient for Method of Standard Additions (MSA) for GFAA less than 0.995	--
S	The reported value was determined by MSA	--
Qualifier		
R ^b	Rejected	--
U ^b	Undetected	--
J ^b	Estimated	--
^a Defined in U.S. EPA 1988. <i>Contract Laboratory Program Statement of Work. Inorganic Analysis, Multi-media, Multi-concentration.</i> ILM04.0. U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Las Vegas, NV. (Flags are assigned by the laboratory). ^b Defined in U.S. EPA 1994. <i>Laboratory Data Validation: Functional Guidelines for Evaluating Inorganic Analyses.</i> U.S. Environmental Protection Agency, Washington, DC.		

23.0 VERIFICATION AND VALIDATION METHODS

23.1 Data Validation Reports

After completing data validation review for a specific analysis type or group of analyses, the review will be summarized in a narrative report that addresses data quality, usability and acceptability in terms of the QAPP requirements. The validation report will be provided to the QA Officer for review in the format of a technical memorandum addressing the following items:

Introduction: This section of the memorandum will provide a short introduction to the project and outlines investigation objectives, DQOs, data quality assessment results, investigation site description, and sampling and analysis summary.

Task and Project Location Name: This section will identify the specific work plan under which the data have been collected and describe the specific project task. Sampling methods will be outlined and the appropriate QAPP referenced. Analytical results will be summarized and data summary tables included.

Calculations: This section of the memorandum will include the procedures used for calculations (if any) and a table indicating the results will be included.

Deviations and Anomalies: Any deviations from the approved QAPP or sampling plan will be listed and include a discussion of the usability of the data.

24.0 RECONCILIATION WITH USER REQUIREMENTS

All data quality issues concerning field sampling efforts, laboratory analysis, data validation, and data reporting will be reviewed by the QA Officer. A data quality assessment will be performed by each entity responsible for using the data to determine whether data generated are consistent with the investigation DQOs for each task. Specific issues to be reviewed include conformance with data quality requirements and overall data completeness, and SWAMP compatibility. Also, if data are found to deviate significantly (several orders of magnitude) from previous analyses or surrounding conditions upon which the sampling program was based, the data may be qualified based on the validator's assessment of the usability of the data for the intended end uses.

ACRONYMS AND ABBREVIATIONS

<	Less than
*	Laboratory qualifier flag – duplicate result outside control limit
%	percent
+	Laboratory qualifier flag – correlation coefficient < 0.995
°C	degrees Celsius
%R	Percent recovery
µg/m ³	micrograms per cubic meter
µg/L	microgram per liter
AA	atomic absorption
ABS	absolute value
AEGL	acute exposure guideline levels
amsl	above mean sea level
amu	atomic mass units
ARARs	Applicable or relevant and appropriate requirements
ASTM	American Society for Testing and Materials
BLM	Bureau of Land Management
B	Boron
Burleson	Burleson Consulting Inc.
Ca	calcium
CaCO ₃	calcium carbonate
Caltest	Caltest Analytical Laboratory
CAM	California Assessment Manual
CBDA	California Bay-Delta Authority
CCB	continuing calibration blank
CCR	California Code of Regulations
CCV	continuing calibration verification
CEC	Cation exchange capacity
CERCLA	Comprehensive Environmental Response, Compensation, & Liability Act
CF	calibration factor
CFm	mean calibration factor
CHHSL	California Human Health Screening Level
cm	centimeter
COC	chain of custody
CoPC	chemicals of potential concern
Cu	copper
CVAFS	cold-vapor atomic fluorescence spectrometry
DFW	California Department of Fish and Wildlife
DI	deionized
DO	dissolved oxygen
DQO	data quality objectives
DTPA	diethylenetriaminepentaacetic acid extraction
DTSC	Department of Toxic Substances Control
DU	decision units

E	laboratory qualifier flag – interference
EDD	electronic data deliverable
EPA	US Environmental Protection Agency
ERP	Ecosystem Restoration Program
Fe	iron
FP	fluoropolymer
GC	gas chromatography
GFAA	graphite furnace atomic absorption
gpm	gallons per minute
Grant	DFW ERP Grant E1596004
HCL	hydrochloric acid
Hg	mercury
HSP	Health & Safety Plan
ICB	initial calibration blank
ICP/MS	inductively coupled plasma/ mass spectrometry
ICS	initial calibration standard
ICV	initial calibration verification
ID	identification
IDL	instrument detection limit
IPR	initial precision and recovery
ITRC	Interstate Technology and Regulatory Council
J	Laboratory qualifier flag - estimated
K	potassium
KCl	potassium chloride
LCS	laboratory control samples
LLICV	low level initial calibration
LOAEL	lowest observed adverse effect levels
M	Laboratory qualifier flag – duplicate injection precision outside control limits
MDL	method detection limit
MEI	McCord Environmental, Inc.
meq	milliequivalents
meq/L	Milliequivalents per liter
Mg	magnesium
mg/kg	milligram per kilogram
mg/L	milligrams per liter
ml	milliliter
mm	millimeter
Mn	manganese
MQO	measurement quality objective
MS	matrix spike
MSA	method of standard additions
MS/MSD	matrix spike/matrix spike duplicate
MSD	matrix spike duplicate
mv	millivolts
N	Laboratory qualifier flag – spike sample results outside control limits
Na	sodium
NA	Not Applicable

Ni	nickel
NIST	National Institute of Standards & Technology
ng/L	Nanograms per liter
ng/g	Nanograms per gram
ng or mg/ m ³	nanograms or milligrams per cubic meter
Nitrate-N	nitrate-nitrogen
OPR	on-going precision and recovery
ORNL	Oakridge National Laboratory
ORP	Oxidation Reduction Potential
OSHA	Occupational Safety & Health Act
PA	preliminary assessment
Pg	picogram
Poly	Polyethylene
PPB	parts per billion
PPM	parts per million
PQL	practical quantitation limits
PRG	preliminary remediation goal
QA	quality assurance
QA/QC	quality assurance/quality control
QAPP	Quality Assurance Project Plan
QC	quality control
R	laboratory qualifier flag - rejected
RL	reporting limit
RPD	relative percent difference
RSD	relative standard deviation
S	laboratory qualifier flag - reported value determined by MSA
SC	specific conductance
Sites	Corona and Twin Peaks Mine project site
SM	Standard Methods for Chemical Analysis of Water and Wastes (EPA 1983)
SOP	standard operating procedure
SW-846	Test Methods for Evaluating Solid Waste/Chemical Methods (EPA 1997)
SWAMP	Surface Water Ambient Monitoring Program
SWRCB	State Water Resources Control Board
TKT	TKT Consulting, LLC
TOC	toxic organic compound
TDS	total dissolved solids
TSS	total suspended solids
Tuleyome	Tuleyome Inc.
U	laboratory qualifier flag - undetected
UC Davis	University of California, Davis
VOA	volatile organic analysis
W	Laboratory qualifier flag - post-digestion spike outside control limits
ZHS	zero headspace
Zn	zinc

REFERENCES

Acute Exposure Guideline Levels (AEGs). 2012. Mercury Vapor Results/Acute Exposure Guideline Levels/US EPA. Available at www.epa.gov/oppt/aegl/pubs/rest184.htm, updated January 11.

A&L Western Laboratories, Inc. 2012. Available at <http://www.al-labs-west.com/>.

Burleson Consulting, Inc. (Burleson). 2012a. Data Quality Objectives for the Corona and Twin Peaks Mines. Prepared for Tuleyome Inc. September.

Burleson. 2012b. Work Plan for Site Characterization for the Corona and Twin Peaks Mine Drainage Treatment Project. September.

Burleson. 2012c. Site Specific Health and Safety Plan (HSP) Checklist for Site Surveys and Sampling Activities. Corona and Twin Peaks Mine Sites. May 8.

California Code of Regulations (CCR). 2012. Title 22. Division 4.5. Environmental Health Standards for the Management of Hazardous Waste. Available at http://www.dtsc.ca.gov/LawsRegsPolicies/Title22/Official_Title22.cfm.

California Department of Fish and Wildlife (DFW). 2011. Grant Number E1596004. Exhibit A, Scope of Work for Corona and Twin Peaks Mine Drainage Treatment Project. Agreement with DFW's Ecosystem Restoration Program (ERP).

California Environmental Protection Agency. 2005. Use of California Human Health Screening Levels (CHHSLs) in Evaluation of Contaminated Properties. Table 1. California Human Health Screen Levels for Soil and Comparison to Other Potential Environmental Concerns. January, 2005. Available at www.calepa.ca.gov/brownfields/documents/2005/CHHSLsGuide.pdf.

California Regional Water Quality Control Board, Central Valley Region. 2003. A Compilation of Water Quality Goals. August.

CLS Laboratories (CLS). 1997. Letter (with attachments) Addressed to Bill Croyle of the California Regional Water Quality Control Board, Central Valley Region Regarding Analytical Results. August 29.

Environmental Protection Agency (EPA). 1983. U.S. Environmental Protection Agency. Standard Methods for the Examination of Water and Wastewater.

EPA. 1988. Contract Laboratory Program Statement of Work. Inorganic Analysis, Multi-media, Multi-concentration. ILM04.0. U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Las Vegas, NV.

EPA. 1994. Laboratory Data Validation: Functional Guidelines for Evaluating Inorganic Analyses.

EPA. 1997. Solid Waste (SW) 846 Test Methods for Evaluating Solid Waste/Chemical Methods. U.S. Environmental Protection Agency, Washington, DC.

EPA. 1998. Method 1630. Methyl Mercury in Water by Distillation, Aqueous Ethylation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry. EPA-821-R-02-019. August. Available at http://water.epa.gov/scitech/methods/cwa/metals/mercury/upload/2007_07_10_methods_method_mercury_1630.pdf.

EPA. 2001. Appendix to Method 1631, Total Mercury in Tissue, Sludge, Sediment, and Soil by Acid Digestion and BrCl Oxidation. EPA-821-R-01-013. January.

EPA. 2002. Method 1631, Revision E: Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry. August. Available at http://water.epa.gov/scitech/methods/cwa/metals/mercury/upload/2007_07_10_methods_method_mercury_1631.pdf.

EPA. 2009. Regional Screening Levels for Chemical Contaminants. Prepared by Oak Ridge National Laboratory, UT Battelle for the Department of Energy. December revision. Available online at: <http://epa-prgs.ornl.gov/chemicals/index.shtml>. U.S. Environmental Protection Agency.

Gavlak, R.G. et al. 2003. Soil, Plant and Water Reference Methods for the Western Region, 2nd Edition. Prepared by R.G. Gavlak, D.A. Horneck, R.O. Miller, J. Kotuby-Amacher. Available at [www: http://isnap.oregonstate.edu/WERA_103/Soil_Methods.htm](http://isnap.oregonstate.edu/WERA_103/Soil_Methods.htm)

Interstate Technology and Regulatory Council (ITRC). 2012. Incremental Sampling Methodology. ISM-1. Washington, D.C: ITRC, Incremental Sampling Methodology Team. Web-based Guidance Document. Available at [www. itrcweb.org/ism-12](http://www.itrcweb.org/ism-12).

MFG, Inc. 2002. Technical Report Former Twin Peaks Mine, Napa County, California. August 16.

Slotton, D.G., S.M. Ayers, and R.D. Weyand. 2008. UC Davis QAPP for Mercury Bioaccumulation Monitoring for the California Bay-Delta Authority (CBDA). August 15.

Slotton, D.G. S.M. Ayers. 2013. Corona/Twin Peaks Mine Remediation Project: 2012 Baseline Stream Biota Mercury Monitoring. April 11.

Slowey, Aaron J; Rytuba, James J; Hothem, Roger L; May, Jason T. 2007. Mercury at the Oat Hill Extension Mine and James Creek, Napa County, California: Tailings, Sediment, Water, and Biota, 2003-2004. U.S. Geological Survey: Menlo Park, CA.

State Water Resources Control Board (SWRCB). 2008. Surface Water Ambient Monitoring Program (SWAMP) Electronic Template for SWAMP-Comparable Quality Assurance Project Plans. Prepared by George Nichol and Emilie Reyes, DWQ. Version 2.0. November 24.

SWAMP. 2015. SWAMP Data Management Plan. January 26. Available at http://www.waterboards.ca.gov/water_issues/programs/swamp/tools.shtml.

UC Davis and McCord Environmental. 2013. Corona and Twin Peaks Mine Drainage Treatment Remediation Project – Year 1 Water Quality Results Summary. November 12.

U.S. Bureau of Land Management (BLM). 2004. Technical Note 390 revised: Risk Management Criteria for Metals at BLM Mining Sites. October.

U.S. Department of the Interior, Bureau of Mines (USBM). 1965. Mercury Potential of the United States, Part 1 of 2. Information Circular 8252. 200 pp.
http://mines.us.gov/DigitalLibrary/usbm_ic/

U. S. Geological Survey (USGS). 2007. Mercury at the Oat Hill Extension Mine and James Creek, Napa County, California: Tailings, Sediment, Water, and Biota, 2003-2004. Open File Report 2007-1132. Prepared by Aaron J. Slowey, James J. Rytuba, Roger L. Hothem, and Jason T. May.

Western States Laboratory Proficiency Testing Program. 1995. Soils and Analytical Methods. Version 2.00. From Plant, Soil and Water Reference Methods for the Western Region, 1994. Prepared by R.G. Gavlak, D.A. Horneck, and R.O. Miller. WREP 125.

Appendix A

UC Davis Mercury Bioaccumulation Monitoring for the California Bay-Delta Authority Quality Assurance Project Plan and Standard Operating Procedures

Quality Assurance Project Plan (QAPP) for

UC Davis Mercury Bioaccumulation Monitoring for the California Bay-Delta Authority (CBDA)

STANDARD OPERATING PROCEDURES (SOPs)

Appendix Contents

	<u>Page Number</u>
UCD SOP #1: Field Sampling and Handling Techniques for Fish and Invertebrates	2
UCD SOP #2: Dry Weight Sample Preparation Techniques for Aquatic Invertebrate and Fish Samples, Prior to Mercury and Other Analyses (plus fresh/wet weight preparation for large fish)	13
UCD SOP #3: Analysis of Total Mercury Using the Perkin-Elmer Flow Injection Mercury System (FIMS)	28
UCD SOP #4: Analysis of Organic (Methyl) Mercury Using the Perkin- Elmer Flow Injection Mercury System (FIMS)	46
UCD SOP #5: Stable Isotope Analysis for Plant and Animal Tissue	66
LABORATORY ORGANIZATION CHART	71

UC Davis SOP #1:
Field Sampling and Handling Techniques
for Fish and Invertebrates

Last revised by Shaun Ayers
August 15, 2008

Originated by Shaun Ayers and Darell Slotton

Dept. Of Environmental Science and Policy
University of California, Davis
One Shields Avenue
Davis, CA 95616

1.0 SCOPE AND APPLICATION

- 1.1 This SOP describes the techniques currently used by the UC Davis Mercury Group to collect, sort, and preserve aquatic insects, clams, crayfish, small fish, and large fish from streams, lakes, and estuaries. These techniques have evolved since 1985 (refs).
- 1.2 Sample labeling, sample transport, sorting, note taking, and initial sample storage will be explained for each sample type.

2.0 SUMMARY OF METHOD

- 2.1 Collection of aquatic samples will occur using the most efficient method available and in as close a proximity to the stated sampling site as possible. Aquatic insect samples are typically field sorted into clean glass vials with Teflon-lined caps. All other samples are placed in clean Ziploc bags for transport to the lab. All samples are labeled with site, date, and sample type.
- 2.2 After samples are collected, they are placed into a cooler with ice for transport back to the lab.
- 2.3 Within 48 hours of collection, samples that have not been field cleaned and sorted will be cleaned and sorted in the lab. Detailed notes should be taken on the type, size, and number of sample organisms collected at each site. Samples that will not be processed immediately should be frozen.
- 2.4 Alternatively, samples can be cleaned and processed in the field and placed directly on dry ice for freezing. Small fish shall be frozen in water. Invertebrates should be sorted, cleaned, extraneous water removed, and placed into pre-weighed vials prior to freezing.

3.0 INTERFERENCES and/or LIMITATIONS

- 3.1 For each site, the quality and quantity of samples collected is limited by the availability of sample organisms, by the weather and water conditions, and by the skill and experience of the collectors.
- 3.2 Contaminant levels in similar sample organisms collected at a site may vary depending upon variables such as individual age, feeding habits, habitat preferences, and residence time at the sampling site. Composite and/or replicate individual sampling of multiple individuals collected from varying intra-site habitats is used to mitigate this variability.
- 3.3 In areas with sediments highly contaminated with mercury, samples run for total mercury could be contaminated by surficially clinging debris on the sample organisms. Special care should be taken to clean and purge samples collected from highly contaminated sites.

4.0 EQUIPMENT and SUPPLIES

- Kick screen
- Pre-weighed 20 ml glass vials with Teflon caps
- Ceramic lined bowls for streamside sorting and cleaning
- Teflon lined forceps (2)
- Sharpie and regular pen
- Field notebook
- Waders and wading boots
- Plastic ice chest(s)
- Ice (and/or dry ice)
- Daypack
- Ziploc bags (quart and gallon size)
- Heavy duty plastic bags (30 gallon)

- Seines (various mesh, length, and depth configurations)
- Gill nets (various mesh sizes and configurations)
- Set lines
- Rods and reels
- Throw nets
- Boat (for setting nets)
- Backpack electro-shocker
- Dip nets
- Fish measuring board
- Pesola scales
- Leatherman tool
- Plastic tubs
- Scientific Collecting permits
- Crayfish traps
- Canned dog food (crayfish bait)
- Sardines (crayfish bait)

5.0 PROCEDURES

5.1 Aquatic Insect Sampling

- 5.1.1 Aquatic insect samples are primarily collected from riffle habitat using a kick screen. Riffle substrate is disturbed by one sampler and sample organisms flow with the current into the screen, held by a second sampler. The kick screen is positioned perpendicular to the flow downstream of the area disturbed. Kick-screen sampling may be supplemented with sampling of submerged vegetation by sweeping various hand-held nets through the vegetation.
- 5.1.2 Once sample organisms have been collected into the screen or hand net, move to the shore and begin removing individuals, by type, into separate ceramic-lined holding bowls containing stream water. Invertebrates are differentiated both by functional feeding level and taxonomic group (normally this corresponds to the genus level) and sorted with stainless steel or Teflon-lined forceps.
- 5.1.3 If adequate sample organisms cannot be collected from one sub-site, collections should be made from other locations as close to the stated sampling site as possible. Enough sample organisms should be collected to create one or more multi-individual composites of each sample type of interest. Replicate samples will consist of similar but unique multi-individual composites of a given sample type, each collected into separate vials and processed independently. Typically, an n of 20 will be used as a minimum for each composite.
- 5.1.4 When sufficient numbers of a given sample type have been assembled, they are then carefully cleaned at streamside, by shaking each individual in the holding water and removing into successively cleaner water baths as necessary. Repeat until no visible debris remains clinging to the sample organisms.
- 5.1.5 For each sample type, measure the range of insect lengths and estimate average length if different from the approximate midpoint of the size range. Record in field book.
- 5.1.6 Line up pre-weighed receiving vials that will correspond to the replicate composites to be prepared. Keep vials in order by placing them into a Styrofoam tube rack or similar stable platform.
- 5.1.7 The insects should then be counted as they are placed into pre-weighed 20 ml vials. A final rinse is performed during transfer into vials. If multiple composites of a given insect type are needed (for the generation of statistical confidence intervals), then individuals should

be placed one at a time into each of the multiple vials, taking care to keep the insect sizes similar across the set of vials.

- 5.1.8 Before sealing vials, remove excess water by inverting the vials over laboratory tissues, being sure to do this consistently across all vials. The objective is to seal the vials with only fresh, wet weight sample organisms inside. Fresh/wet weight of the insects can then be determined in the laboratory through differential weighing. Average individual weight is obtained by dividing combined weight by number of individuals.
- 5.1.9 Record in field book the following information, at minimum, for each sample: site, insect type, unique sample number, vial number, date, insect length range, and number of individuals in the composite.
- 5.1.10 Each vial should also be labeled with vial number, site, insect type, sample number, date, insect length range, and number of individuals in the composite.
- 5.1.11 Place all of the vials from a site into a clean, double Ziploc bag and place this package on ice for transport back to the laboratory. Alternatively, freeze samples directly in the field by placing on dry ice. Maintain samples frozen in laboratory freezer prior to drying and homogenization.
- 5.1.12 Transfer all appropriate sample information from field book into the computer database.

5.2 Clam Sampling

- 5.2.1 Clam samples will be collected by hand or with a variety of rake-like implements, from the sandy sediments at a site. Wash the sediment from the clam and place directly into a Ziploc bag. Ideally, collect 20-30 clams in a size range appropriate to the project.
- 5.2.2 Label the Ziploc bag with site and date. Record sample information in field book: site, date, number of clams, size range.
- 5.2.3 Place bagged clam samples in a cooler with ice for transport back to the lab.
- 5.2.4 Within 24 hours of sampling, clam samples should be washed with DI or tap water in a plastic colander, and placed into plastic tubs for purging. All of the clams from a site will be placed into one tub. Separate tubs will be used for each site.
- 5.2.5 Fill each tub with de-chlorinated water until clams are deeply submerged. Twice a day (morning and evening) change the water in the tub. During each water change, wash the clams and the tub of accumulated debris. Visually (and manually if a question) inspect the clams regularly to make sure they are all alive. Purge the clams for 72-96 hours; at that time no more debris should be being expelled from the clams.
- 5.2.6 After purging has been completed, place the clams in new, properly labeled Ziploc bags, fill the bags partially with tap or DI water, sufficient to surround all of the clams, remove air from bag, and freeze until sample preparation and drying procedures can be undertaken.
- 5.2.7 Replicate samples from each site will consist either of unique individuals or of similar but unique multi-individual composites, each processed independently in the sample preparatory phase.

5.3 Crayfish Sampling

- 5.3.1 Crayfish samples are collected directly through electro-shocking, or by using baited, modified minnow traps set for from several hours to overnight. Traps should be set from a boat or from shore over rocky substrate in slowly flowing water. Return following the appropriate time period for retrieval.
- 5.3.2 Chose individuals to retain for analysis based on needs of the project.
- 5.3.3 Place all of the crayfish to be retained from a site into a large Ziploc bag labeled with site and date. Note quantity and relative size of the samples in a field notebook. Place the bag on ice for transport back to the lab.
- 5.3.4 Within 24 hours of collection, wash the crayfish with tap or DI water in a plastic colander.
- 5.3.5 Place the samples into a new Ziploc bag labeled with site and date. Add sufficient water to cover all of the crayfish, remove air from the bag, and freeze. Store samples in the freezer until sample preparation and drying procedures can be initiated.
- 5.3.6 Crayfish can be prepared and analyzed either individually or as multi-individual composites.
- 5.3.7 For multi-individual composite analyses, replicates from each site will consist of similar but unique multi-individual groupings of crayfish, each processed independently in the sample preparatory phase.

5.4 Small Fish Sampling

- 5.4.1 Small fish may be collected using several different techniques depending on the physical characteristics of the collection site and the availability of sample organisms. Commonly used small fish collection techniques include seining, baited minnow traps, backpack electrofishing and boat-based electrofishing. *Prior to electrofishing, all field personnel must be properly trained to ensure all safety procedures are followed.*
- 5.4.2 Collection of fish should occur within as close proximity as possible to the stated sampling site.
- 5.4.3 The objective is to obtain sufficient replication for statistical treatment of mean mercury levels. Typically replicate, similar individuals are collected and analyzed of each target species at each site, with an n of 10-30. Alternatively, if samples are extremely prevalent, replication can be achieved through the collection of three to six composites, each composed of multiple individuals (n = 5+ per composite), all within a size range that is common among the project sites.
- 5.4.4 All small fish samples from the site should be separated, by species, into Ziploc bags labeled with the site and date, water added to the bag and frozen on dry ice. Fish samples can also be placed on regular ice for transport and separation in the laboratory. In this case water should not be added to the bag. Alternatively composites can be separated in the field. Depending on project specific objectives, measurement of individual fish total length may occur in the field or back in the lab after thawing. The method of field fish preservation and measurement will be clearly stated in the project objectives and used consistently throughout that project.
- 5.4.5 Within 24 hours of sample collection, wash off all clinging debris with clean water within a stainless steel or plastic colander. Arrange fish by species and size. As the fish are separated, take detailed notes about the species, numbers, and sizes of the fish. Separate different species of fish into different Ziploc bags once clean and label with site, date, and species. Add sufficient water to surround all individuals. Freeze the fish in water in sealed ziplock bags with airspace removed.
- 5.4.6 Alternatively, fish can be cleaned, separated, and measured directly in the field. Cleaning will consist of removal of surficial clinging material using a technique of multiple transfers, with shaking, into successively cleaner water baths. The fish are then placed in properly labeled Ziploc bags with clean water sufficient to surround all individuals, airspace is

removed, and the bags are sealed and frozen on dry ice until they can be transferred to a laboratory freezer to await processing.

5.5 Large Fish Sampling

- 5.5.1 Large fish may be collected using several different techniques depending on the physical characteristics of the collection site and the availability of sample organisms. Commonly used large fish collection techniques include seines of various mesh sizes and configurations, gill nets of various mesh sizes and configurations, baited set lines, angling, and backpack electrofishing. *Prior to electrofishing, all field personnel must read the operations manual for the Smith-Root Model 15-D backpack electrofisher for proper safety instruction.*
- 5.5.2 Collection of large fish should occur within as close a proximity as possible to the stated sampling site.
- 5.5.3 The type and size of fish collected will be project specific but, in general, large fish are collected across a range of sizes so that a size vs mercury relationship can be established. Large fish muscle mercury is typically analyzed for individual fish rather than composites, if at all possible. Live fish can be weighed and measured in the field if necessary. Detailed field notes should be kept about species and size of individuals caught.
- 5.5.4 Fish that will be sacrificed for analysis should be euthanized quickly with a sharp blow by wooden club, placed in Ziploc or large plastic bags labeled with site and date. For non-destructive muscle mercury sampling of live fish in the field, see UCD biological sample preparatory SOP. Take scale samples for aging if appropriate.
- 5.5.5 Bagged fish should be placed on ice for transport back to the lab. Within 24 hours, fish should be dissected for analytical muscle samples directly or preparation of intermediate fillet chunks (5.5.6 below). Alternatively, whole fish may be frozen directly in the field, using dry ice. Prior to freezing, wrap each fish in plastic wrap to minimize freezer burn; seal in labeled Ziploc bag with air removed.
- 5.5.6 Alternatively a piece of the fillet can be removed from the “shoulder” region of the fish above the lateral line and just posterior of the head

and gill cover. Leave the skin on this piece of fillet. The piece should be wrapped in plastic wrap to minimize freezer burn and then sealed in a Ziploc bag with air removed. Transfer to freezer or onto dry ice. This process should be completed within 24 hours of collection. Remove a large enough piece so that a fresh, uncompromised subsample of the fillet can be extracted from the center. This should be at least 3 cm x 3 cm and the full depth of the fillet.

- 5.5.7 Label the bag containing the fillet piece with fish type, location, date, and size metrics.

6.0 QUALITY ASSURANCE and CORRECTIVE ACTIONS

- 6.1 Field replicate samples will be collected at a frequency of at least 1 in 10 to assess the variability of contaminants in sample organisms. If that variability is found to be unacceptably high, the sampling and compositing protocol may be varied to mitigate the effect. Multiple individuals or composites of a given insect or fish type shall serve as field replicates.
- 6.2 Field sampling should only be done by individuals fully trained in the above techniques and familiar with the taxonomy of aquatic invertebrates and fish.

7.0 REFERENCES

- Axler, R.P., J.E. Reuter, D.G. Slotton and C.R. Goldman. 1988. Heavy metal distribution and water quality aspects of a newly impounded gold mining reservoir. *Verh. Internat. Verein. Limnology*. (23) 968-975.
- Puckett, H.M. and B.H. van Buuren. 2000. *Quality Assurance Project Plan for the CALFED Project: An assessment of ecological and human health impacts of mercury in the Bay-Delta watershed*, available at: <http://loer.tamug.tamu.edu/calfed/QA.htm>.
- Reuter, J.E., D.G. Slotton, S.M. Ayers, and C.R. Goldman. 1999. Environmental monitoring for mercury in water, sediment, and biota in Davis Creek and Davis Creek Reservoir. *Report for Yolo County*. 183 pp. (similar reports from 1985-1998).
- Slotton, D.G. 1991. Mercury bioaccumulation in a newly impounded Northern California reservoir. *Doctoral dissertation, University of California, Davis, December 1991*. 363 pp.
- Slotton, D.G., S.M. Ayers, J.E. Reuter, and C.R. Goldman. 1995. Gold mining impacts on food chain mercury in northwestern Sierra Nevada streams. *Technical Completion Report for the University of California Water Resources Center, Project W-816*, August 1995, 46 pp.

- Slotton, D.G., J.E. Reuter, and C.R. Goldman. 1995. Mercury uptake patterns of biota in a seasonally anoxic northern California reservoir. *Water, Air, and Soil Pollution*, 80:841-850.
- Slotton, D.G., S.M. Ayers, J.E. Reuter, and C.R. Goldman. 1997. Cache Creek watershed preliminary mercury assessment, using benthic macro-invertebrates. *Final Report for the Central Valley Regional Water Quality Control Board and the National Science Foundation*, June 1997, 36 pp.
- Slotton, D.G., S.M. Ayers, and J.E. Reuter. 1998. Marsh Creek watershed mercury assessment project: Third year (1997) baseline data report with 3-yr review of selected data. *Report for Contra Costa County*, June 1998, 62 pp.
- Slotton, D.G., S.M. Ayers, J.E. Reuter, and C.R. Goldman. 1999. Lower Putah Creek 1997-1998 mercury biological distribution study. *Report for the Dept. of Environmental Health and Safety, University of California, Davis*, February 1999, 59 pp.
- Slotton, D.G., S.M. Ayers, T.H. Suchanek, R.D. Weyand, and A.M. Liston, C. Asher, D.C. Nelson, and C. Asher. 2002. The effects of wetlands restoration on the production and bioaccumulation of methylmercury in the Sacramento-San Joaquin Delta, California. *Report for the CALFED Bay-Delta Agency*. 76 pp.
<http://loer.tamug.tamu.edu/calfed/FinalReports.htm>
- Slotton, D.G., S.M. Ayers, T.H. Suchanek, R.D. Weyand, and A.M. Liston. 2004. Mercury bioaccumulation and trophic transfer in the Cache Creek watershed of California, in relation to diverse aqueous mercury exposure conditions. *Report for the California Bay-Delta Authority*. 137 pp.
<http://loer.tamug.tamu.edu/calfed/FinalReports.htm>

UC Davis SOP #2:
**Dry Weight Sample Preparation Techniques
for Aquatic Invertebrate and Fish Samples,
Prior to Mercury and Other Analyses
(plus fresh/wet weight preparation for large fish)**

Last revised by Shaun Ayers
August 15, 2008

Originated by Shaun Ayers and Darell Slotton

Dept. Of Environmental Science and Policy
University of California, Davis
One Shields Avenue
Davis, CA 95616

1.0 SCOPE AND APPLICATION

- 1.1 This SOP describes the techniques currently used by the UC Davis Mercury Group to convert samples of various aquatic organisms to a dry powder form that is well homogenized, consistent in terms of moisture content, and is stable at room temperature for long term archiving. Preparation steps for fish muscle wet/fresh weight analysis are also described. These techniques are based on methods developed and revised from 1985 to the present (see references).
- 1.2 Specifically this SOP will outline techniques for preparation (for total mercury, methyl mercury, and stable isotope analysis) of aquatic invertebrate, clam, and small fish whole body composite samples, as well as individual muscle samples from crayfish and large fish (on either a wet or dry weight basis).
- 1.3 The technique for determining percent moisture in these samples will be described in the event that data on wet tissue concentrations is needed or wet/dry weight conversion factors need to be determined for a given sample type.

2.0 SUMMARY OF METHOD

- 2.1 Appropriate individual or composite samples of fresh/wet invertebrates, clams, or small fish are assembled and measured, extraneous surface moisture is removed, and samples are placed into pre-weighed containers for sequential weighing and drying. For individual muscle samples from crayfish or large fish, appropriate weighing and measuring procedures are followed on whole individuals, followed by excision of muscle samples for direct wet weight analysis and/or for dry processing and sample archiving. Direct wet analysis utilizes small, app. 200 mg chunks weighed directly into digestion vessels. For dry weight analysis, larger muscle pieces are placed into pre-weighed containers for sequential weighing and drying. All samples for dry analysis are dried at 55

°C in a recirculating laboratory drying oven for at least 48 hours. Prior to sample homogenization, the drying oven is shut off and the samples are allowed to cool and equilibrate moisture content for 4-6 hours. Small aquatic arthropod samples (insects, myscids, cladocerans, etc.) are homogenized using teflon coated instruments and stored in the same glass containers in which they were dried. All other dry samples (shrimp, small fish, molluscs, fish and crayfish muscle) are homogenized using a modified coffee grinder which is cleaned between samples. Powdered, homogenized samples are transferred to glass vials with teflon lined closures and labeled with information on site, collection date, type of organism, and composite number or size.

3.0 INTERFERENCES and/or LIMITATIONS

- 3.1 This SOP describes techniques for homogenization of composite or individual samples. The quality of the analytical data is limited by the ability to generate adequately homogenized samples. Care should be taken throughout the procedure to insure full homogenization. Small samples (<100 mg dry) should be avoided because they typically do not homogenize well.
- 3.2 Steps should be taken to avoid contamination of samples by contact with unclean lab equipment and residue from other samples. Samples should also never come in direct contact with uncovered lab counters or unclean hands.

4.0 EQUIPMENT and SUPPLIES

- 1 3/8 oz aluminum weighing tins
- 20 ml glass screw thread sample vials with teflon caps
- Large aluminum tins
- Krups coffee grinder with plexiglass insert to decrease volume
- Brush to clean coffee grinder
- Lab countertop paper
- Paper towels
- Kimwipes
- Teflon lined forceps
- Teflon lined Spatula
- Lab notebook

- Sharpie and regular pen
- Labelling tape
- Stainless steel scalpel with extra blades
- Aluminum foil
- Balance accurate to ± 0.001 g
- Recirculating laboratory drying oven
- DI water
- 10% nitric acid washing solution in a squirt bottle
- Plastic ruler
- Pesola scales
- Plastic tub

5.0 PROCEDURES

5.1 Aquatic Invertebrate (Insects, Myscids, Zooplankton, etc) Sample Preparation

- 5.1.1 Remove pre-cleaned and sorted aquatic invertebrate composite samples (in their vials) from the freezer and arrange the vials by site and sample type in large aluminum tins.
- 5.1.2 After thawing to room temperature, remove the labeled vial caps and place, Teflon lining down, on a tray covered with clean lab countertop paper. Put the tray with the caps on it in a safe place where it will not get disturbed. Cover the caps with aluminum foil.
- 5.1.2 If samples were placed into pre-weighed vials, obtain weight of vial plus sample (minus lid). Make sure the outside of the vial is dry.
- 5.1.3 Place the aluminum tins containing the glass vials and samples into the drying oven and dry for at least 48 hours at 55 °C.
- 5.1.4 4-6 hours prior to sample homogenization, turn off the drying oven and allow samples to cool to room temperature with the door closed.
- 5.1.5 Re-weigh sample vials (again minus lids) for dry weight determinations.
- 5.1.6 Re-join the labeled caps with the appropriately labeled vials. Continue to keep the samples arranged by site and sample type.
- 5.1.7 Homogenize each sample in its vial directly by crushing the dried invertebrates repeatedly with the flat end of a teflon-lined spatula or similar lab tool. Continue the procedure until individual sample pieces are approximately the size of sand grains or less. Clean the spatula between samples by vigorously wiping with a Kimwipe until no visible sample residue is left on the spatula. To further minimize the possibility of cross contamination, homogenize samples relative to their approximate relative mercury concentration, moving from low concentration to high concentration (i.e. low to high trophic levels within each site). Rinse homogenizing implements with DI water and dry thoroughly with further Kimwipe cleaning between sites.
- 5.1.8 Transfer the appropriate sample information data from the field and laboratory notebooks into the computer database. Total and Methyl mercury runs can then be scheduled, and chains of custody can be created for samples that will be sent to other labs.

5.2 Clam Sample Preparation

- 5.2.1 Remove ziplocked, purged clam samples from the freezer and allow bags to thaw in a water bath.
- 5.2.2 Once thawed, clams should be removed from their bags, set out on clean lab countertop paper, and arranged by size so that compositing decisions can be made. The types of composites chosen is project specific and can vary depending on the sizes and number of clams collected from each sampling site. In general, if site-to-site comparisons are being made, similar sized clams are composited together from each site, with an emphasis on trying to prepare 3-6 matched multi-individual composites within a size range that is common among all of the sites to be sampled. Alternatively, individuals can be analyzed.
- 5.2.3 Once compositing decisions have been made, record in the lab notebook the site, date of sampling, composite identification, number of clams in the composite, and the maximum width of the smallest and largest clam in the composite, measured to the nearest millimeter.
- 5.2.4 With a stainless steel scalpel pry open each clam shell and cut out the entire soft body. Place all of the clam bodies from the composite in a pre-weighed (to $\pm 0.001\text{g}$), sequentially numbered aluminum weighing tin. Acid wash the scalpel and rinse thoroughly with DI between composites. Replace the scalpel blade when moving to a new site. Record the tin number, empty tin weight, and composite data in the lab notebook.
- 5.2.5 Zero the balance and obtain the weight of the tin + wet sample. The sample wet weight (individual or composite) can later be determined by subtracting the empty tin weight from the weight of the tin + sample. For composites, the average individual weight can later be determined by dividing the composite wet weight by the number of individuals in the composite.
- 5.2.6 Arrange the weighing tins in larger aluminum tins and place in drying oven. Dry samples for at least 48 hours at 55 °C.
- 5.2.7 4-6 hours prior to sample homogenization, turn off the drying oven and allow samples to cool to room temperature with the door closed.
- 5.2.8 Remove samples from drying oven and weigh the tin + sample to $\pm 0.001\text{g}$. Determine the average dry body weight by subtracting the tin weight from the tin + dry sample weight and dividing the sample weight by the number of individuals in the composite. Record this data in the lab notebook.

- 5.2.9 Homogenize the clam composite samples in the modified coffee grinder. Place all of the dried clams from a given composite into the grinder and pulse the on/off switch until the composite sample is a fine powder.
- 5.2.10 Transfer the powder into a 20 ml vial with Teflon lined cap. (Pre-label the vial with site, date, sample type, and composite number.)
- 5.2.11 Between composite samples, clean the grinder with a fine bristle brush and wipe out with Kimwipes until no visible sample residue is left in the grinder. To minimize the possibility of cross contamination, homogenize samples relative to their approximate relative mercury concentration, moving from low concentration to high concentration sites.
- 5.2.12 Transfer the appropriate sample information data from the field and laboratory notebooks into the computer database. Total mercury runs can then be scheduled, and chains of custody can be created for samples that will be sent to other labs.

5.3 Small Fish Sample Preparation

- 5.3.1 Remove small fish samples, frozen in ice blocks in Ziploc bags, from the freezer and allow the sealed bags to thaw in a room temperature water bath.
- 5.3.2 Once thawed, small fish should be rinsed and set out on paper towels overlying clean lab countertop paper. If the fish are to be run individually, arrange by size, choose individuals to analyze and, for each, measure total length, remove extraneous surface moisture by light blotting with laboratory tissue, and proceed to 5.3.5 for sequential weighing and drying. All information should be written down on a lab worksheet or in a lab notebook.
- 5.3.3 If the fish are to be composited, arrange in order from smallest to largest within each sample to facilitate compositing. The types of composites chosen may be project specific and can vary depending on the sizes and number of small fish collected from each sampling site. In general, similar sized small fish are composited together, with an emphasis on the preparation of 3-6 matched multi-individual composites from each sampling in a size range that is common among all of the sites/dates to be sampled.
- 5.3.4 Once size decisions have been made and the fish have been separated into composites, record in the lab notebook the fish species, site, collection date, composite identification, number of fish in the composite, and the total length of the smallest and largest fish in the composite, measured to the nearest millimeter. Additionally note mean length if different than approximate midpoint of size range. Remove extraneous surface moisture by blotting fish lightly with laboratory tissues.
- 5.3.5 Sequentially number a weighing tin and record the number along with the other sample data in the lab notebook. Zero the balance and weigh the empty tin to $\pm 0.0001\text{g}$ and record. Zero the balance again and place the individual fish or multi-individual composite into the tin, weigh and record. The sample wet weight (individual or composite) can later be determined by subtracting the empty tin weight from the weight of the tin + sample. For composites, the average individual fish weight can later be determined by dividing the composite wet weight by the number of individuals in the composite.
- 5.3.6 Arrange the weighing tins with samples in a larger aluminum holding tin and place in drying oven. Dry samples for at least 48 hours at 55 °C.
- 5.3.7 4-6 hours prior to sample homogenization, turn off the drying oven and allow samples to cool to room temperature with the door closed.

- 5.3.8 Remove dry fish samples from drying oven, zero balance, and re-weigh the tin + sample to ± 0.001 g. Record in lab notebook. The dry sample weight can later be determined by subtracting the empty tin weight from the tin + sample dry weight.
- 5.3.9 Homogenize the small fish individual or composite samples in the modified coffee grinder. Place the dry individual or all of the dried individuals from a composite into the modified grinder and pulse the on/off switch until the composite sample is a fine powder.
- 5.3.10 Transfer the powder into a clean, pre-labeled 20 ml vial with Teflon lined cap. (Pre-label the vial with information on sample type, site, date, and composite number).
- 5.3.11 Between samples, clean the grinder with a fine bristle brush and wipe out with Kimwipes until no visible sample residue is left in the grinder. To further minimize the possibility of cross-contamination, homogenize samples in order of increasing size and presumed mercury concentration if relevant.
- 5.3.12 Transfer the appropriate sample information data from the field and laboratory notebooks into the computer database. Total and/or methyl mercury runs can then be scheduled, and chains of custody can be created for samples that will be sent to other labs.

5.4 Crayfish Sample Preparation

- 5.4.1 Remove crayfish samples from the freezer in their sealed bags and thaw in a room temperature water bath. When thawed, rinse individual crayfish in tap or DI water.
- 5.4.2 For each site, arrange crayfish from smallest to largest on clean lab countertop paper. For each individual, measure carapace length (from tail joint to shelf on rostrum) to the nearest millimeter, and weigh the whole individual to ± 0.001 g. Note any missing claws/legs that would affect weight. Determine sex of each individual if apparent. Enter the above information into the lab notebook under the appropriate site and date.
- 5.4.3 Starting with the smallest crayfish (within a given species and site), remove the tail and extract the tail meat out onto a laboratory tissue. With a scalpel, cut away attached gut and excise gut running the length of the tail meat along the dorsal side. Clean the scalpel between individuals by wiping with a Kimwipe and rinsing thoroughly with DI water. Replace the scalpel blade between sites. Place the tail meat from each crayfish in its own sequentially numbered, pre-weighed tin. Note the tin number in the lab notebook along with the rest of the information for that crayfish.
- 5.4.4 Zero the balance and obtain the weight of the tin + wet sample. The sample wet weight can later be determined by subtracting the empty tin weight from the weight of the tin + sample.
- 5.4.5 Arrange the weighing tins in larger aluminum holding tins and place in drying oven. Dry samples for at least 48 hours at 55 °C.
- 5.4.6 4-6 hours prior to sample homogenization turn off the drying oven and allow samples to cool to room temperature with the door closed.
- 5.4.7 Remove samples from drying oven and weigh each tin + sample to ± 0.001 g. Determine the dry sample weight by subtracting the tin weight from the tin + dry sample weight. Record this data in the lab notebook.
- 5.4.8 Homogenize the dried crayfish muscle samples individually (or as composites) in the modified coffee grinder. Pulse the on/off switch until the sample is a fine powder.
- 5.4.9 Transfer the powder into a 20 ml vial with Teflon lined cap. Label the vial with information on sample type, site, collection date, and size of the individual (whole body grams / millimeters carapace length).

- 5.4.10 Between samples, clean the grinder with a fine bristle brush and wipe out with laboratory tissues until no visible sample residue is left in the grinder. To further minimize the possibility of cross contamination, homogenize samples relative to their mercury concentration, moving from low concentration to high concentration.
- 5.4.11 Transfer the appropriate sample information data from the field and laboratory notebooks into the computer database. Total mercury runs can then be scheduled, and chain of custodies can be created for samples that will be sent to other labs.

5.5 Large Fish Sample Preparation

- 5.5.1 Remove whole frozen fish from the freezer and allow to thaw, one sample set at a time. Alternatively, utilize fresh, refrigerated (iced) fish. For field non-destructive sampling technique with live fish, proceed to 5.5.5. Rinse fish with tap or DI water and arrange by size.
- 5.5.2 If only a piece of the fillet has been kept, thaw and proceed to 5.5.4.
- 5.5.2 Measure fish length to ± 1 mm with a ruler or fish measuring board, and weigh the fish to ± 5 grams using an appropriate Pesola scale. Record this information in the lab notebook, under the appropriate site, date, and species heading.
- 5.5.3 Make a shallow, U-shaped incision in the dorso-lateral (“shoulder”) region of the fish. Pull back the skin to reveal the flesh. With a clean scalpel, cut out a section of fillet approximately 5 cm long, 3 cm wide, and 1-2 cm deep. Be careful to avoid bones and only cut out muscle tissue. Place the fillet chunk on a laboratory tissue and cut away the fatty layer that was in contact with the skin. Clean the scalpel between individuals by wiping with a Kimwipe and rinsing thoroughly with DI water. Replace the scalpel blade when it starts to get dull and between sites.
- 5.5.4 If a sample is going to be run for mercury on the fresh/wet tissue, dissect out a “raisin-sized” chunk (~ 0.200 g) of tissue, remove extraneous surface moisture by patting lightly on a laboratory tissue, and place directly into a tared digestion tube on the balance. Record the sample weight to ± 0.001 g. Proceed to analysis.
- 5.5.5 Alternatively, for non-destructive muscle sampling of live large fish in the field: Working quickly after removing an individual fish from the live well, measure length to ± 1 mm on a fish measuring board, weigh the fish to ± 5 grams using an appropriate Pesola scale, and record this information in the field book, along with site, date, and species information (already noted). Remove several scales from the dorso-lateral target area as needed and, using a scalpel with a fresh stainless steel blade (or a clean core-biopsy tool), dissect out a small plug of fillet muscle that is approximately 5 mm x 5 mm in area at the skin surface and approximately 7-10 mm in depth into the tissue (remove a proportionately larger plug for split or spike samples). Release fish. Place the file muscle plug on a clean laboratory tissue and dissect off the end containing skin and fatty layer, leaving a clean, raisin-sized chunk(s) (~ 0.200 g each) of tissue as used in lab-prepared fish muscle samples. Remove extraneous surface moisture by patting lightly on a laboratory tissue, and place directly into a pre-weighed digestion tube. Seal the tube

carefully with a fresh piece of Parafilm and screw digestion cap on tightly. Maintain tubes in sealed ziplock bags on ice. Obtain tube + sample weights within 48 hours of collection. Bring sealed tubes to room temperature in a lab hood, allowing time for the evaporation of any surficial moisture on the tubes. Remove cap and Parafilm seal, tare balance, weigh tube + sample, and record the weight to ± 0.001 g. Re-cap the tube and prepare for mercury analysis. NOTE: Potential temporal variability in tube weights has been traced to the caps, with the glass tubes themselves remaining constant. Therefore, most accurate sample weights are obtained by performing critical weighings without the caps. Blank tubes are brought into the field together with the sample tubes and weighed before and after the sampling, identical to the sample-containing tubes. The mean difference in initial vs final blank tube weights (n=3-7) has never exceeded 0.001 g to date. In the event that a variation from initial weight averaging ≥ 0.002 g is noted in the blanks, which are weighed prior to the sample tubes, the entire set of tubes would be allowed to stabilize longer, which would presumably remove the variation. If the variation remained, the sample-containing tube weights would be adjusted by that amount.

- 5.5.6 (Returning to lab-dissected, larger muscle samples): To dry the remaining piece of fillet (for homogenous dry sample generation; e.g. for methyl:total Hg ratio, stable isotopes, and/or archiving), transfer to a sequentially numbered, pre-weighed tin and record the number along with the other information about the fish.
- 5.5.7 Zero the balance and obtain the weight of the tin + wet sample. The sample wet weight can later be determined by subtracting the empty tin weight from the weight of the tin + sample.
- 5.5.8 Arrange the weighing tins in the larger aluminum holding tins and place in drying oven. Dry samples for at least 48 hours at 55 °C.
- 5.5.9 4-6 hours prior to sample homogenization turn off the drying oven and allow samples to cool to room temperature with the door closed.
- 5.5.10 Remove samples from drying oven and weigh each tin + sample to ± 0.001 g. Determine the dry sample weight by subtracting the tin weight from the tin + dry sample weight. Record this data in the lab notebook.
- 5.5.11 Homogenize the dried fish fillet chunks in the modified coffee grinder. Pulse the on/off switch until the sample is a fine powder.
- 5.5.12 Transfer the powder back into a pre-labeled 20 ml vial with Teflon lined cap. Label the vial with information on site, date, sample type, and size of the individual (grams whole fresh weight / millimeters length).

- 5.5.13 Between samples clean the grinder with a fine bristle brush and wipe out with laboratory tissues until no visible sample residue is left in the grinder. To further minimize the possibility of cross contamination, homogenize samples relative to their mercury concentration, moving from presumed low concentration to high concentration.
- 5.5.14 Transfer the appropriate sample information data from the field and laboratory notebooks into the computer database. Total mercury or other analytical runs can then be scheduled, and chains of custody can be created for samples that may be sent to other labs.

5.6 Percent Moisture

- 5.6.1 In all of the above sample preparation procedures, percent moisture data can be derived by measuring the difference between sample weight before and after going through the drying procedure. However, for the most accurate results, care should be taken to not let the samples dry out prior to the initial weighing. Freezing tends to dry out the samples. Freezing small fish, crayfish, or clams in water largely eliminates this problem, as does the wrapping of fillet pieces in plastic wrap. Subsequent drying on the laboratory bench is minimized by working rapidly with exposed samples, working with one sample set at a time, and keeping individuals moist until immediately prior to weighing. Samples directly field-collected into pre-weighed vials (invertebrates) or digestion tubes also circumvent the potential differential drying issue if handled carefully.

6.0 QUALITY ASSURANCE and CORRECTIVE ACTIONS

- 6.1 For total and methyl mercury analyses, split samples will be run at a minimum rate of 1 per 20 samples. Differences between split samples measure analytical variability plus the variability that may result from poor homogenization. If high RPDs ($\geq 20\%$) are found in samples with concentrations $>10x$ the detection level, then homogenization protocols should be reviewed as a possible cause.
- 6.2 At the beginning of each new project, and periodically throughout the project, laboratory equipment and laboratory supplies that come in contact with samples will be tested for significant contamination. This will be done by rinsing equipment with wash acid and running the rinse water for total mercury. Paper towels, Kimwipes, lab countertop paper, etc. will also be digested and run for total mercury. If any significant contamination hazard is found, corrective action will be taken. Thus far, the materials routinely utilized provide no opportunity for significant contamination of solid environmental samples. Similarly, no cross contamination has ever been noted (through spike recovery results, splits, etc.) from biota homogenization procedures that remove all visible material from instruments before moving to the next sample.
- 6.3 Whenever a new sample matrix or a particularly large sample is run through the drying procedure, it will be checked to make sure the currently stated drying time is adequate.

7.0 REFERENCES

- Axler, R.P., J.E. Reuter, D.G. Slotton and C.R. Goldman. 1988. Heavy metal distribution and water quality aspects of a newly impounded gold mining reservoir. *Verh. Internat. Verein. Limnology*. (23) 968-975.

- Puckett, H.M. and B.H. van Buuren. 2000. *Quality Assurance Project Plan for the CALFED Project: An assessment of ecological and human health impacts of mercury in the Bay-Delta watershed*, available at: <http://loer.tamug.tamu.edu/calfed/QA.htm>.
- Reuter, J.E., D.G. Slotton, S.M. Ayers, and C.R. Goldman. 1999. Environmental monitoring for mercury in water, sediment, and biota in Davis Creek and Davis Creek Reservoir. *Report for Yolo County*. 183 pp. (similar reports from 1985-1998).
- Slotton, D.G. 1991. Mercury bioaccumulation in a newly impounded Northern California reservoir. *Doctoral dissertation, University of California, Davis, December 1991*. 363 pp.
- Slotton, D.G., S.M. Ayers, J.E. Reuter, and C.R. Goldman. 1995. Gold mining impacts on food chain mercury in northwestern Sierra Nevada streams. *Technical Completion Report for the University of California Water Resources Center, Project W-816*, August 1995, 46 pp.
- Slotton, D.G., J.E. Reuter, and C.R. Goldman. 1995. Mercury uptake patterns of biota in a seasonally anoxic northern California reservoir. *Water, Air, and Soil Pollution*, 80:841-850.
- Slotton, D.G., S.M. Ayers, J.E. Reuter, and C.R. Goldman. 1997. Cache Creek watershed preliminary mercury assessment, using benthic macro-invertebrates. *Final Report for the Central Valley Regional Water Quality Control Board and the National Science Foundation*, June 1997, 36 pp.
- Slotton, D.G., S.M. Ayers, and J.E. Reuter. 1998. Marsh Creek watershed mercury assessment project: Third year (1997) baseline data report with 3-yr review of selected data. *Report for Contra Costa County*, June 1998, 62 pp.
- Slotton, D.G., S.M. Ayers, J.E. Reuter, and C.R. Goldman. 1999. Lower Putah Creek 1997-1998 mercury biological distribution study. *Report for the Dept. of Environmental Health and Safety, University of California, Davis*, February 1999, 59 pp.
- Slotton, D.G., S.M. Ayers, T.H. Suchanek, R.D. Weyand, and A.M. Liston, C. Asher, D.C. Nelson, and C. Asher. 2002. The effects of wetlands restoration on the production and bioaccumulation of methylmercury in the Sacramento-San Joaquin Delta, California. *Report for the CALFED Bay-Delta Agency*. 76 pp.
<http://loer.tamug.tamu.edu/calfed/FinalReports.htm>
- Slotton, D.G., S.M. Ayers, T.H. Suchanek, R.D. Weyand, and A.M. Liston. 2004. Mercury bioaccumulation and trophic transfer in the Cache Creek watershed of California, in relation to diverse aqueous mercury exposure conditions. *Report for the California Bay-Delta Authority*. 137 pp.
<http://loer.tamug.tamu.edu/calfed/FinalReports.htm>

UC Davis SOP #3: Analysis of Total Mercury Using the Perkin-Elmer Flow Injection Mercury System (FIMS)

Last revised by Shaun Ayers
August 14, 2008

Originated by Darell Slotton, Ron Weyand, and Shaun Ayers

Dept. Of Environmental Science and Policy
University of California, Davis
One Shields Avenue
Davis, CA 95616

- 1. SCOPE AND APPLICATION-** This SOP outlines sample digestion and subsequent analysis of samples for determination of mercury in aquatic tissues. The analysis is performed by the Perkin-Elmer Flow Injection Mercury System, Model 100 (better known as the FIMS 100). The analysis method is based on EPA Method 245.6, as well as methodology developed within the UC Davis Mercury Group.
- 2. METHOD DETECTION LIMITS-** MDL= 0.001 ug/g Hg (both wet and dry samples, 6/14/07 data)
- 3. WORKING RANGE-** 0.001-1.875 ug/g (dry samples), 0.001-0.430 ug/g (wet samples)- (These ranges are based on a 1-ml sample loop. These ranges can be extended by use of smaller sample loops, allowing for analysis of high-level mercury samples).
- 4. SUMMARY OF METHOD-** In order to be analyzed by the cold vapor atomic absorption technique, total sample mercury needs to be in the +2 oxidation state. For this to occur, tissue samples of various aquatic species are oxidized in reaction tubes by digestion with a nitric acid/sulfuric acid mixture, followed by further oxidation with a potassium permanganate/potassium persulfate solution. Both oxidations are carried out in a 95-degree water bath. After reduction of any remaining permanganate by hydroxylamine hydrochloride, the samples are brought to volume in the reaction tubes with deionized water. The prepared samples are allowed to flow into the FIMS, along with an acidic carrier solution. The mercury in the sample digests is reduced to elemental mercury by reaction with stannous chloride in a reaction manifold. The gaseous elemental mercury is stripped from the sample solution by argon gas in a gas/liquid separator. The mercury is swept by the gas into a long glass cell that is positioned along the light path of a high-intensity mercury lamp. The sample mercury in the cell absorbs some of the light emitted by the mercury lamp. This decrease in light energy is directly proportional to the amount of mercury in the sample. The FIMS 100 Spectrophotometer is an automated system operating under Microsoft Windows and offers many options for data calculation and reporting.

5. INTERFERENCES-

- 5.1. Any possible reduction interference from sulfide is eliminated by the addition of KmnO_4 . It has been shown that sulfide concentrations as high as 20 mg/L (S as Na_2S) do not interfere with mercury recovery from deionized water.
- 5.2. Samples high in chlorides may require additional KmnO_4 . Free chlorine is produced from chlorides in the sample during the oxidation step. This free chlorine has an absorption line near that of mercury (~253 nm). Addition of excess hydroxylamine hydrochloride to the sample, along with vigorous mixing and shaking of the sample, will purge free chlorine from the sample solution. The reaction tubes will be uncapped at the time of analysis, thus ensuring removal of contaminants from the dead-air space above the sample solutions.
- 5.3. Any interference from certain volatile organic materials (with absorption lines near 253 nm) will be removed when samples are shaken, than uncapped for analysis. This removes contaminants from the dead-air space above the sample.

6. SAFETY-

- 6.1. This analysis employs the use of various hazardous chemicals. All precautions should be taken to avoid exposure to these potentially dangerous chemicals. A lab coat and safety glasses are required at all times when performing this analysis. All digestions, preparation of reagents, and dissipation of waste mercury should be performed in the safety hood.
- 6.2. For specific information on the chemicals used in this analysis, please refer to the Material Safety Data Sheet (MSDS) for each specific chemical. The chemical MSDS will provide information on toxicity, flammability, handling protocol, and clean-up procedures in case of accidental spillage or contact.

7. INSTRUMENTATION/EQUIPMENT-

- 7.1. Perkin-Elmer FIMS 100 Spectrophotometer with the AS-90 Autosampler
- 7.2. Fisher Scientific 10-Liter Hot Water Bath
- 7.3. Ohaus "Precision Advanced" Model GT410 Top-Loading Analytical Balance
- 7.4. Thermolyne Cimarec 2 Hot Plate/Stirrer
- 7.5. Fisher Scientific "Genie 2" Vortex Mixer
- 7.6. Gilsop "Pipetman" Adjustable Pipetor
- 7.7. Eppendorf Repeater Pipetor
- 7.8. Kimble 20 ml glass culture tubes with caps
- 7.9. Various analytical glassware: volumetric flasks, graduated cylinders, beakers, etc.

8. REAGENTS AND STANDARDS-

- 8.1.** Deionized Water (>17 umhos/cm)
- 8.2.** Stock Mercury Solutions- Prepare two separate stock solutions-one for the calibration curve and matrix spikes; the other for laboratory control samples (LCS's). Dry, certified mercuric chloride (HgCl_2) is used in stock solution preparation. Use different brands or lot numbers for the two stock solutions. Keep a log of all standard preparations in the UC Davis Mercury Group "Standard and Solution Preparation" notebook. Prepare as follows:
 - 8.2.1.** Fill a 200 ml volumetric flask to ~50 ml with deionized water. Add 1.0 ml concentrated nitric acid (to acidify and preserve the solution) and mix. Weigh 0.2708g HgCl_2 onto clean weighing paper and transfer to the volumetric. Mix and bring to mark with deionized water to produce a 1000 ug/ml stock mercury standard. Repeat this procedure for the second stock solution. These solutions are stable for two years.
 - 8.2.2.** Prepare a 10 ug/ml working mercury standard by half-filling a 100 ml volumetric with deionized water. Add 0.5 ml concentrated nitric acid and mix. Pipet 1.0 ml of the 1000 ug/ml mercury standard into the volumetric. Mix and bring to mark with deionized water to produce a 10 ug/ml mercury standard. Repeat the procedure for the second working solution. These solutions are stable for two months.
 - 8.2.3.** Prepare a 0.100 ug/ml working mercury standard by half-filling a 100 ml volumetric flask with deionized water. Add 0.5 ml concentrated nitric acid and mix. Pipet 1.0 ml of the 10 ug/ml mercury standard into the volumetric. Mix and bring to mark with deionized water to produce a 0.100 ug/ml mercury standard. Repeat the procedure for the second working solution. These solutions are stable for a period of two weeks after preparation. Be sure to keep solutions tightly closed.
- 8.3.** Concentrated Nitric Acid (HNO_3)- Use only Trace Metal Grade
- 8.4.** Concentrated Sulfuric Acid (H_2SO_4)-Use only Trace Metal Grade
- 8.5.** Concentrated Hydrochloric Acid (HCl)- Use only Trace Metal Grade
- 8.6.** Potassium Permanganate (KmnO_4)- Use only dry, certified reagent labeled "suitable for mercury determination."
- 8.7.** Potassium Persulfate ($\text{K}_2\text{S}_2\text{O}_8$)-Use only dry, certified reagent labeled "suitable for mercury determination."
- 8.8.** Sodium Chloride (NaCl)-Use ACS Reagent Grade or equivalent.
- 8.9.** Hydroxylamine Hydrochloride ($\text{NH}_2\text{OH}\cdot\text{HCl}$)-Use only reagent labeled "suitable for mercury determination."
- 8.10.** Sodium Hydroxide (NaOH) pellets- Use any grade.
- 8.11.** Bromcresol Green/Methyl Red indicator solution- Dissolve 0.1g bromcresol green (sodium salt) and 0.1g methyl red (sodium salt) in 100 ml DI water. One can also use a commercially-prepared solution of these two chemicals.
- 8.12.** Potassium Permanganate/Potassium Persulfate oxidizing solution (5%/5% w/v)- Fill a 1-liter volumetric flask to ~ 700 ml with deionized water (do not use this volumetric flask for any other reagent preparation). Add 50g potassium permanganate and 50g potassium persulfate to the flask. Swirl to

mix. Gently warm and stir the solution on a hot plate/stirrer to help dissolve the reagent crystals. When dissolved, remove from the plate, cool, and bring to mark with deionized water. This solution is stable for one year.

- 8.13.** Sodium Chloride/Hydroxylamine Hydrochloride clearing solution (12%/12% w/v)- Weigh 6g sodium chloride and 6g hydroxylamine hydrochloride and add to a 50 ml volumetric flask. Add a few mls of deionized water and swirl to mix. When crystals are dissolved, bring to mark with deionized water. This solution must be prepared fresh on the day of use.
- 8.14.** 3% Hydrochloric Acid carrier solution- Fill a 2-liter volumetric flask with ~1500 ml of deionized water. Add 60 ml concentrated hydrochloric acid while swirling. Add deionized water and bring to mark. Prepare this reagent fresh on the day of use.
- 8.15.** 1.1% w/v Stannous Chloride reducing solution- Fill a 1-liter volumetric flask with ~ 500 ml deionized water. Add 30 ml concentrated hydrochloric acid while swirling. Weigh and add 11g stannous chloride ($\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$) to the flask and swirl to dissolve the crystals. When dissolved, bring to mark with deionized water. This solution must be prepared fresh on the day of use. Do not use any glassware or utensils that have come into contact with stannous chloride in preparation of other reagents. (Caution: Historically, it has been found that certain package lots of stannous chloride do not allow full recovery of mercury from certified reference materials (CRM's) and samples. Be sure to test each lot of stannous chloride by analyzing replicate samples of the CRMs that are used in the laboratory. If recoveries are not acceptable, do not use the problematic stannous chloride in mercury determinations.)
- 8.16.** 2:1 Sulfuric Acid/Nitric Acid digestion solution- Place one part concentrated nitric acid into an appropriately-sized beaker. While swirling, add two parts concentrated sulfuric acid to the nitric acid in the beaker (caution- this procedure produces a vigorous exothermic reaction). Prepare this solution fresh on the day of use.
- 8.17.** Sodium Hydroxide solution for waste trap- During the course of the analysis of samples, the Hg standards, samples, and reaction chemicals are collected in a large plastic container. This mixture is acidic due to the 3% HCl solutions and the sample/standard digests. Sodium Hydroxide is used to neutralize and destroy this acidic mixture during the analytical run. Any Hg from the digests is also removed in this container due to the reaction with stannous chloride. The Hg is removed from solution and is trapped on a carbon filter. This neutralized analysis waste can then be discarded.
- 8.17.1.** Assemble the waste trap as depicted in Appendix 3.
- 8.17.2.** Remove the rubber stopper. Fill the plastic container with ~ 1 liter of water.
- 8.17.3.** Using equivalent weights of HCl, HNO_3 , and H_2SO_4 , calculate the equivalent weight of NaOH needed to neutralize all sample and reagent solutions. Weigh the NaOH and add to the container of water. Swirl to dissolve.

- 8.17.4. Place the container on the stir plate and begin stirring the solution. Add a few drops of the bromocresol green/methyl red indicator solution. The solution will now be blue/green in appearance.
- 8.17.5. Replace the stopper, making sure that the container is airtight. Turn on the laboratory air valve in order to produce bubbling in the waste trap. Adjust the bubble rate to ~ 2 bubbles/second. The waste trap is now ready for use.
- 8.17.6. During the course of the analysis, monitor the color of the waste solution in the trap. If it begins to turn red, add more NaOH pellets until the solution is once again blue/green. Check the pH of the solution at the end of the analysis with pH paper. Adjust the pH of the solution with NaOH pellets or dilute HCl as needed to produce a neutral-pH solution. Once the solution is at neutral pH, it can be discarded.

9. SAMPLE DIGESTION (always performed in a fume hood)-

- 9.1. Prepare an Analysis Worksheet containing all pertinent sample information. This worksheet is prepared by computer under the Microsoft Excel format. A copy of the full printout of the analysis worksheet is attached at the end of this SOP as Appendix 1. The full worksheet is normally not printed and used throughout the analysis. Only data such as culture tube numbers, sample weights, and full post-digested tube weights are recorded on a partial hard-copy of the analytical worksheet. This copy of the worksheet accompanies the samples through the digestion and analytical procedures. All data, including that on the hardcopy, is transferred to the analytical worksheet in the computer, thus allowing the computer to calculate analytical parameters and results.
- 9.2. Set up the appropriate number of clean 20-ml culture tubes in one or more tube racks for the digestion.
- 9.3. Number the tubes with a permanent black marking pen. Also, number the culture tube caps with the corresponding number using an electric engraver.
- 9.4. Weigh each empty tube (including the cap) and record the weight on the worksheet. Alternatively, the tubes may be weighed in advance and the weights entered into a computer database. The database can then be accessed at the time of analysis to obtain the needed tube weights.
- 9.5. Turn on the hot water bath and set the “Temperature Set” knob to ~ 95°. Allow the bath to heat up while preparing the samples for digestion. Once the bath has reached temperature, use a certified thermometer to measure the bath water temperature. Adjust the “Temperature Set” knob as necessary to produce a water bath temperature of $95 \pm 1.0^\circ\text{C}$. Record the thermometer temperature and the digital readout from the water bath in the “UC Davis Mercury Group Equipment Manual.”
- 9.6. Add samples, standards, and Quality Assurance (QA) samples to the appropriate culture tubes as follows:
 - 9.6.1. For dry samples and Certified Reference Materials (CRM’s), place a culture tube (without cap) on the Ohaus analytical balance and tare its

weight. Mix the sample in its container by rolling and/or shaking. Place ~ 50 mg of dry sample into the culture tube and replace on the balance. Record the sample weight on the worksheet. Cap and place the tube back into the rack.

- 9.6.2.** For the standard curve, pipet the appropriate amount of standard directly into the culture tube. The standard mercury amounts currently used for the standard curve are as follows:

<u>Standard #</u>	<u>Hg Conc.</u>	<u>Mls 0.100 ug/ml Hg Std added</u>
1	0 ng	0
2	10 ng	0.10
3	20 ng	0.20
4	40 ng	0.40
5	60 ng	0.60
6	90 ng	0.90
7	120 ng	1.20
8	150 ng	1.50

- 9.6.3.** In order to assess the accuracy and precision of the analytical method, different types of Quality Assurance (QA) samples are analyzed along with the samples. The type of QA samples analyzed and their frequencies are as follows:

- 9.6.3.1. Matrix Spike-** A matrix spike is analyzed once every ten samples. The matrix spike is prepared by adding a known amount of mercury to the sample matrix being analyzed. Currently, 50 ng of mercury is added to the matrix to prepare the matrix spike. This amount is approximately the average of the sample concentrations being found in UC Davis Mercury Group sample analysis. The mercury standards used in standard curve preparation are used in matrix spike preparation. The unit for the matrix spike level is ug/g.
- 9.6.3.2. Sample Duplicate or Split-** A sample duplicate or split is analyzed once every ten samples. The duplicate is an “extra” analysis of the same sample. The duplicate analysis is performed on the same sample used for the matrix spike preparation.
- 9.6.3.3. Laboratory Control Standard (LCS)-** An LCS is analyzed once every twenty samples. The LCS is prepared by adding a known amount of mercury to deionized water, which is then carried through the entire analytical procedure. The source of mercury for the LCS must be of a different lot number or manufacturer than that of the matrix spike mercury source. Most LCS concentrations are within the range of 10-50ng. The level is reported as ug/L.
- 9.6.3.4. Reagent Blank-** A reagent blank is analyzed once every twenty samples. The reagent blank is prepared by using deionized water

in place of sample matrix, then carried through the entire analytical procedure. The units of reporting are ug/L.

9.6.3.5. Certified Reference Material (CRM)- Three different CRM types, in duplicate, are analyzed per analytical run. The three types of CRMs are the TORT-2 (lobster hepatopancreas) and DOLT-2 (dogfish liver) materials furnished by the National Research Council of Canada, and NIST 2976 Mussel tissue furnished by the National Institute of Standards and Technology. These are sample matrixes that have been characterized and certified for mercury content. Each CRM is carried through the analytical procedure and the percent recoveries of the analyte mercury are determined.

- 9.7.** Pipet an amount of deionized water, equal to the volume of mercury standard added to the matrix spikes (usually 0.50 ml), to each of the tubes containing dry sample, as well as the reagent blanks. This assures that all sample tubes contain the same volume through the digestion process.
- 9.8.** Add 1.0 ml of the 2.1 sulfuric acid/nitric acid digestion solution to each culture tube. Cap and mix or vortex to assure complete mixing.
- 9.9.** Place sample rack(s) containing all tubes into the 95°C water bath. Place the water bath cover over the water bath and digest the sample tubes for one hour. Begin the timing of the digestion when the water bath returns to 95°C.
- 9.10.** While the samples are digesting, warm and stir the potassium permanganate/potassium persulfate oxidizing solution (see 8.10.) on a hot plate/stirrer to re-dissolve any crystals that may have formed upon storage.
- 9.11.** After one hour of digestion, carefully remove the cover from the water bath (avoid dripping any water onto the samples). Remove the samples from the water bath and allow to cool. Replace the cover on the water bath to maintain its 95°C temperature.
- 9.12.** When cool, uncapped the sample tubes (the contents may be under pressure-use gloves and caution). Set the caps aside. Pipet one ml of the warmed-and-stirred potassium permanganate/potassium persulfate oxidizing solution into each tube (this may produce a reaction in the tube, so add with caution). Stir or vortex the samples to mix. Add another one-ml aliquot of the oxidizing solution to each tube (for a total of two ml of oxidizing solution per tube). Stir or vortex each tube to ensure complete mixing.
- 9.13.** Place the uncapped tubes back into the 95°C water bath and carefully, without dripping, place the cover on the water bath. Allow the samples to digest for two hours. Begin timing of the digestion when the water bath returns to 95°C. Periodically check the samples to ensure that the potassium permanganate does not change to a ruddy brown color. This would suggest a sample high in organic content. Potassium permanganate must be kept in excess throughout the digestion process. If necessary, add an additional 0.5 ml of the oxidizing solution until the color of the solution remains the purple permanganate color.
- 9.14.** After two hours of digestion, carefully remove the cover from the water bath (avoiding any water dripping into the samples). Remove the samples from the

water bath and allow to cool. The samples can now be capped and stored for no more than two days, or the samples can be prepared for analysis as follows:

- 9.15. If samples have been stored, remove the caps and put them aside. Add 0.5 ml of the sodium chloride/hydroxylamine hydrochloride clearing solution to each tube. Swirl or vortex to mix. This neutralizes any excess potassium permanganate present in the tube. The sample solutions should become colorless and, depending on the sample matrix, reasonably clear.
- 9.16. Fill each tube with fresh deionized water up to the level demarcated by the top of each culture tube white label. This corresponds to approximately 16-17 ml total sample volume.
- 9.17. Cap each tube and shake the tubes to ensure proper mixing of the deionized water and digested-sample liquid (if mixing is not done properly, inconsistent sample results will occur upon analysis).
- 9.18. Make sure each tube is clean and dry by wiping with a clean tissue or Kimwipe.
- 9.19. Weigh each clean, dry sample tube on the top-loading balance. Record each weight on the analysis worksheet. Determine the weight (and, subsequently, the volume in mls) of each sample by subtracting the empty tube-and-cap weight from the full tube-and-cap weight (this is automatically done by computer upon transferring all data to the analysis worksheet in the computer). The samples are now ready for analysis.

10. SET-UP OF THE PERKIN-ELMER FIMS 100 ANALYTICAL SYSTEM FOR SAMPLE ANALYSIS (for further information, consult the various manuals supplied by Perkin-Elmer regarding all parameters of the FIMS analytical systems. These are listed at the end of this SOP as Appendix 2)

- 10.1. At least one hour before analysis, turn on the FIMS system by depressing the “on” switch on the front of the Dell computer, as well as depressing the “on-off” switch on the front of the FIMS 100 unit, which is set below the AS-90 Autosampler. This allows the mercury lamp within the FIMS unit to warm and stabilize before analysis.
- 10.2. Open the gas cylinder main valve (on top of the cylinder) and the gas outlet valve (small black knob located on the left side of the gas valve assembly). Use the large black valve located on the front of the gas valve assembly to adjust the gas pressure to 360 kPa.
- 10.3. Upon start-up of the computer, Windows will initiate the operating system.
- 10.4. Click “Cancel” when the “Enter Network Password” window appears.
- 10.5. Click “Exit” when the “Program Diskettes” window appears. This brings the screen to the default Desktop configuration.
- 10.6. Double-click on the “AA Winlab Analyst” icon to initiate the Perkin-Elmer AA Winlab, V. 2.50, Instrument Control software.
- 10.7. Select a workspace by clicking on the “Use a custom-designed workspace” icon, then choose the file name by selecting the “sma.fms” file. Click on “OK” to open the workspace. The screen will then display all the pertinent

windows used in the analytical procedure. The windows displayed are as follows:

- 10.7.1.** Automated Analysis
 - 10.7.2.** Examine Peak
 - 10.7.3.** FIAS Control
 - 10.7.4.** Examine Calibration
 - 10.7.5.** Results
 - 10.7.6.** AA Winlab toolbar
- 10.8.** Click “File,” highlight “New” with the cursor, then click “Sample Info File.” This displays the Sample Information Editor window. This window is used to enter and save sample information into a file used by the FIMS system.
- 10.8.1.** Enter the description of the analytical set (Example: 11Delta99, 01Sed99, etc.).
 - 10.8.2.** Enter the batch ID, if necessary (this parameter is usually left blank).
 - 10.8.3.** Enter the initials of the analyst.
 - 10.8.4.** Enter the sample volume as “16.000.” This is the approximate volume of the samples.
 - 10.8.5.** Click on the arrow next to “Volume Units” and choose “ml.” This is the unit of volume used for the samples.
 - 10.8.6.** Click on the arrow next to “Weight Units” and choose “g.” This is the unit of weight used for the samples.
 - 10.8.7.** Sample descriptions will now be entered in the “Sample ID” column. Click on the “Sample ID” box corresponding to the autosampler location for the first sample (not calibration standard). Use the scroll bar on the far right side to move to the required location. Currently, most samples analyzed by the UC Davis Mercury Group begin at autosampler location “17.” Enter the sample description.
 - 10.8.8.** Tab right to the “Sample Weight” column. Enter the sample weight in grams.
 - 10.8.9.** Fill in the “Sample ID” and “Sample Weight” columns for all the samples.
 - 10.8.10.** Double-click on the gray box labeled “Sample Units.” The “Sample Units Column Fill” window will appear. Select the units that the computer will use to report the final sample results by clicking on the small circle next to “Weight/Weight Units.” Click on “ug/g.”
 - 10.8.11.** Move to the Location Range area and select the autosampler location for the first sample of the analytical run (Start), then select the autosampler location for the last sample of the analytical run (End). Click “OK.” The units of “ug/g” should appear in the “Sample Units” column of each sample.

- 10.8.12.** The final sample volume for each sample is not exactly 16.000 mls, as was entered in 10.8.4., above. Due to this fact, a dilution factor must be entered into the computer to be used by the computer in correctly calculating the final result. This factor for each sample will be entered in the “User Dilution” column. Calculate the dilution factor for each sample by taking its final weight or volume (see 9.19.) and dividing by “16.” This number, usually over 1.0, is entered for each sample in the “User Dilution” column.
 - 10.8.13.** Enter any necessary remarks for each sample in the “Remarks” column.
 - 10.8.14.** Click on “File,” then click on “Save.” The “Save As” window appears. Give the sample batch, that was just entered, a file name. The file name cannot be more than eight characters in length. After entering the file name, click “OK.” The file is now saved in the computer software.
 - 10.8.15.** Close the Sample Information Editor window.
- 10.9.** Choose an analytical method for the samples by double-clicking on the box directly below the “Method” title in the “Setup” tab of the Automated Analysis window. This displays the “Open Method” window. Highlight the method required for analysis in the “Name” column. For most samples, use the “1 ml method” (if samples are high in mercury content, use of a 0.5 ml sample loop and corresponding “0.5 ml method” may be required). Use the scroll bar on the far right of the window, if necessary, to move to the appropriate method. Click “OK” to select the highlighted method. The Automated Analysis window will now display the selected method in the “Method” column.
 - 10.10.** Click on the “MethEd” icon in the toolbar. The “Method Editor” window for the selected method is displayed. This window allows the analyst to modify certain parameters regarding the selected method. There are six different subgroups (shown as tabs along the bottom of the window) within the window that show various areas of modification. The subgroups and the required settings for the various parameters within each subgroup are as follows:
 - 10.10.1. Inst.-** These are important instrument settings for the selected method. The normal settings for the “1 ml method” are as follows:
 - 10.10.1.1. Method Desc.-** 1 ml injection 0-250 ng Hg
 - 10.10.1.2. Wavelength-** 253.7
 - 10.10.1.3. Read Time (sec)-** 25.00
 - 10.10.1.4. Read Delay (sec)-** 0.0
 - 10.10.1.5. BOC Time (sec)-** 2
 - 10.10.1.6. Type-** Atomic Absorption
 - 10.10.1.7. Measurement-** Peak Height
 - 10.10.1.8. Smoothing (pts)-** 9

10.10.2. Calib.- This tab has three pages of calibration parameters that can be modified. The settings for the “1 ml method” are as follows:

10.10.2.1. Equation , Units, & Replicates

10.10.2.1.1. Equation- Zero Intercept: Linear

10.10.2.1.2. Max. Decimal Places- 3

10.10.2.1.3. Max. Significant Figures- 4

10.10.2.1.4. Calibration- ug/L

10.10.2.1.5. Sample- ug/L

10.10.2.1.6. Replicates- Choose the “fixed” option and set to “2.” Click on the “Set” button. In the “Samples” field, select the first option and set the replicate number to “1.” In the “Calibration Solutions” field, set the replicate number to “1.” In the “QC Samples” field, select the first option. When complete with all fields, click “OK.”

10.10.2.2. Standard Concs (page name)- This page is used to input the standard curve identifications, concentrations, and autosampler locations.

10.10.2.2.1. The “ID” and “A/S Loc.” (autosampler location) columns will contain the following identifications: Calib. Blank = Calib Blank, A/S loc. = 9; Reslope Std. = Reslope, A/S loc. = (not used); Reagent Blank = Reagent Blank, A/S loc. = (not used); Standard 1 = 10 NG, A/S loc. = 10; Standard 2 = 20 NG, A/S loc. =11; Standard 3 = 40 NG, A/S loc. = 12; Standard 4 = 60 NG, A/S loc. = 13; Standard 5 = 90 NG, A/S loc. =14; Standard 6 = 120 NG, A/S loc. = 15; Standard 7 = 150 NG, A/S loc. =16

10.10.2.2.2. The “Conc.” Column changes for each analytical run. The standard concentrations in ug/L are entered here. To determine these values, divide the standard concentrations in ng by their appropriate final volumes (see 9.19.). The resulting numbers are the standard concentrations in ug/L. Enter the value for each standard in its appropriate box.

- 10.10.2.3. Auto-Sampler (page name)-**
 - 10.10.2.3.1. Tray Type-** Tray B
 - 10.10.2.3.2. Frequency-** Between Samples
 - 10.10.2.3.3. $\sqrt{\quad}$ - Wash only if sample “Concentration” > “3” ug/L**
 - 10.10.2.3.4. Location of Wash Solution-** 0
 - 10.10.2.3.5. Cycles:** Wash for “1” cycles
 - 10.10.2.3.6. Use FIAS steps “1” to “1” as programmed**

- 10.10.3. FIAS-** This tab lists important pump and valve parameters that can be edited. Settings for the “1 ml method” are as follows:
 - 10.10.3.1. Sample Volume (ul)-** 1000
 - 10.10.3.2. Use Amalgam-** No
 - 10.10.3.3. The “Flow Injection Program” columns should be set as follows:** Prefill-Time (sec) = 10, Pump 1 Speed = 120, Valve = Fill; 1- Time (sec) = 15, Pump 1 Speed = 120, Valve = Fill; 2- Time (sec) = 25, Pump 1 Speed = 120, Valve = Inject, highlight “read step.”
 - 10.10.3.4. Steps to Repeat-** “1” to “1.”
 - 10.10.3.5. Number of Repeats-** 0

- 10.10.4. Checks-** This tab lists various checks on calibration and analysis measurements that can be edited. Settings for the “1 ml method” should all be highlighted “No.”
- 10.10.5. QC-** This tab lists various QC calculations and procedures that can be performed by the computer. It contains four different pages. The UC Davis Mercury Group does not use this tab. Keep all fields blank or in default settings to disregard.
- 10.10.6. Options-** This tab lists various optional parameters that can be edited. The settings for the “1 ml method” are as follows:
 - 10.10.6.1. The “Results Display/Printed Log” field should be set to “Default.”**
 - 10.10.6.2. The “Peaks to Save” field should be set to “None.”**

- 10.10.7.** When all fields have been set, click “File,” then “Save.” All parameters are saved for the “1 ml method.” Close the “Method Editor” window.

- 10.11.** Move to the Automated Analysis window and click “Browse” next to the field labeled “Sample Information File.” The File List window will appear. Highlight the analysis file that was produced in 10.8.14. above, then click

- “OK.” This will set up the FIMS system to analyze the selected analytical set. The file name should now appear in the “Sample Information File” field.
- 10.12.** In the same Automated Analysis window, click “Browse next to the field labeled “Results Data Set Name.” The “Choose Results Data Set” window will appear. Type in the result name for the analytical set in the “Result Name” field. This will be name for the sample results for this analytical set (it is usually given the same name as the Sample Information File). Click “OK” to save this name. The name should now appear in the “Results Data Set Name” field.
 - 10.13.** In the Automated Analysis window, move to the “During Analysis” field and highlight “Save Data” and “Print Log.” In the “Off After Analysis” field, highlight “Pump.”
 - 10.14.** At the bottom of the Automated Analysis window, click the “Analyze” tab to display the buttons and fields necessary to start/stop the analysis.
 - 10.15.** Move to the “FIAS Control” window and the “Manual Operation” field. Make sure the “Valve Fill/Inject” settings for Pumps 1 and 2 are set to “120.”
 - 10.16.** The computer and software parameters required for automated sample analysis are now set.
 - 10.17.** Install the pump tubing on the pump magazines as follows:
 - 10.17.1.** An illustration as to the different types of pump tubing and their installation locations can be found in the manuals listed at the end of this SOP as Appendix 2. All tubing and valve openings must be free of any obstructions. Flush obstructed valve openings with pressurized deionized water and replace any tubing that is blocked.
 - 10.17.2.** Remove the pump magazines from their prospective pivots. The pump tubing can be installed with the magazines in place, but the tubing is easier to install without being attached to the pivots.
 - 10.17.3.** Gently pull the tubing and hook the tensioned tubing in the appropriate pump magazine between two of the collars on the pump tube.
 - 10.17.4.** Slide each pump magazine onto the appropriate pivot above or below the pump. Remember that solution flow is from left to right for pump magazines placed above the pump roller, and right to left for pump magazines placed below the pump roller.
 - 10.17.5.** Turn the pressure adjustment lever until the end of the pressure screw locks into the notch at the back of the pump magazine.
 - 10.18.** Be sure that all tubing connections are secure and tight on both the FIAS Valve and Gas-Liquid Separator. If any tubing is damaged or kinked, replace with new tubing identical in part number to the original tubing. For the “1 ml method,” install the proper tubing as follows:
 - 10.18.1.** Install the 1-ml sample loop on the FIAS Valve. Be sure that both ends are installed tightly.

- 10.18.2.** Install tube # 198097 to the two manifold blocks of the Gas/Liquid Separator. Be sure that both ends do not kink and that they are installed tightly.
 - 10.18.3.** Install a 500 ul sample loop between the center manifold block and the main Gas/Liquid Separator block. This is the chemical reaction coil. Be sure that both ends do not kink and that they are installed tightly.
- 10.19.** Place the ends of the carrier and reductant tubes into separate beakers filled with deionized water. Place the ends of the sample and carrier waste tubes into a large plastic waste container.
 - 10.20.** Fill the plastic wash beaker, located within the AS-90 Autosampler sample tray, with deionized water and place below the sample probe on the autosampler. Click on the “Move Probe Up/Down” button on the Automated Analysis window. This will move the sample probe down into the wash beaker filled with deionized water.
 - 10.21.** Click on the “Pump 1” button in the “Manual Operation” field of the “FIAS Control” window. This will initiate the pump roller to turn. Deionized water should flow through the carrier, reductant, and sample probe tubes.
 - 10.22.** After initiation of the pump roller, check the flow rates of the various tubes as follows:
 - 10.22.1.** Fill a 10 ml graduated cylinder to the 10 ml mark with deionized water.
 - 10.22.2.** Set a timer for a time period of one minute.
 - 10.22.3.** While the pump roller is turning, insert the dry end of the tubing being tested into the 10 ml cylinder. As soon as the end of the tube touches the water, start the timer.
 - 10.22.4.** Allow the deionized water to flow through the tube for one minute. At the end of the timed minute, remove the tube from the cylinder.
 - 10.22.5.** Note the flow rate in mls/minute. The acceptable flow rates for the various tubes are as follows: Carrier tube: 9-11 mls/min; Reductant tube: 5-7 mls/min; Sample Probe tube: 8.5-10.5 mls/min. If the flow rates are not appropriate, replace faulty tubing.
 - 10.22.6.** Place the ends of the tubes back into their prospective beakers.
 - 10.23.** Turn off the pump roller by clicking on the “Pump 1” button in the “Manual Operation” field of the “FIAS Control” window. Adjust the gas flow to ~75-80 m³/min by turning the black knob directly beneath the gas flow gauge on the front of the FIMS unit. This will correspond to ~ 50-60 m³/min when the pump roller is activated. The flow of gas through the instrument will terminate if the FIMS system is idle for more than 10 minutes. The gas flow automatically resumes again upon initiation of the pump roller.
 - 10.24.** Allow the instrument to rinse with deionized water for ~5-10 minutes.
 - 10.25.** Prepare the Carrier and Reductant solutions (see 8.12. and 8.13.). Place the ends of the Carrier and Reductant tubes into their appropriate solutions.

- 10.26. Discard the deionized water in the plastic wash beaker after sample probe removal. Fill with Carrier solution and re-introduce the sample probe into the beaker.
- 10.27. Allow all solutions to flow through the instrument for ~ five minutes.
- 10.28. Immediately before sample analysis, stop the pump roller. Attach the tubing from the FIMS Cell to the top of the Gas/Liquid separator block. Be sure the tube is dry and installed tightly. Unscrew and remove the top of the Gas/Liquid Separator and blot off any moisture that may have accumulated on the PTFE membrane within the Separator (this will also need to be done every 8-10 samples throughout the sample analysis). Screw the top back on to the Separator. The FIMS System is now ready for sample analysis.

11. AUTOMATED SAMPLE ANALYSIS BY THE PERKIN-ELMER FIMS 100 ANALYTICAL SYSTEM

- 11.1. Uncap and place all sample tubes in their proper positions in autosampler Tray B. Sample locations must correspond to those given the samples in the Sample Information File (see 10.8.7. and 10.10.2.2.1.).
- 11.2. Place the plastic wash beaker in autosampler location "0."
- 11.3. Place the filled autosampler tray on top of the AS-90 Autosampler. Be sure the tabs on the bottom corners of the tray "lock" into place on top of the AS-90. This keeps the tray from moving during analysis.
- 11.4. Move to the Automated Analysis window on the computer screen and click on the "Calibrate" button. The FIMS System will automatically initiate the calibration procedure and begin analyzing the mercury standards for the calibration curve. The printer will print the calibration data as the procedure continues. As the calibration proceeds, monitor the various windows on the computer screen. If any data looks unacceptable, re-click on the "Calibrate" button when the autosampler is between samples. This action will stop the analysis. Correct any suspected problems, then re-click on the "Calibrate" button. This will once again initiate the calibration procedure.
- 11.5. When the calibration procedure is completed, highlight the "Examine Calibration" window. Click on "File," highlight "Print," then click on "Window Image." This will tell the printer to print the calibration plot before analysis of the samples occurs.
- 11.6. After examination and acceptance of the calibration data, click the "Analyze Samples" button in the Automated Analysis window. This will automatically initiate the sample analysis mode of the FIMS System. As with the calibration procedure, monitor the computer screen for any anomalies in the sample analysis. If the sample analysis needs to be terminated or interrupted, click on the "Analyze Samples" button. This will interrupt the analysis. When the analysis is set to continue, re-click the "Analyze Samples" button. The "Continuing an Analytical Sequence" window will appear. Select the appropriate selection, then click "OK." This will once again initiate the sample analysis (depending on the parameters chosen in the "Continuing an Analytical Sequence" window).

- 11.7. As the sample analysis continues, the printer will print sample results expressed in both “ug/g” and “ug/L” units. These results can then be inputted to the appropriate reporting format required for expression of results.
- 11.8. Upon completion of sample analysis, the AS-90 Autosampler will move the sample probe to a location above the wash beaker. The pump roller will stop. The printer will print the results for the last samples. Shut down the FIMS 100 System by the following:
 - 11.8.1. Fill the wash beaker with deionized water and click on “Move Probe Up/Down” to lower the sample probe into the water.
 - 11.8.2. Fill two beakers with deionized water and place the ends of the Carrier and Reductant tubes into the water.
 - 11.8.3. Click the “Pump 1” button in the “Manual Operation” field of the “FIAS Control” window. This will initiate the pump roller and deionized water will flow through all of the tubes. Let the tubes rinse for ~ 5-10 minutes.
 - 11.8.4. While the tubes rinse, remove the rubber stopper from the waste trap plastic container. While the waste solution continues to stir, pour all standard and sample digest solutions into the container. Also, pour the SnCl₂ solution into the container. This will destroy and neutralize all acid and mercury from these solutions. Replace the rubber stopper.
 - 11.8.5. Remove the ends of the Carrier and Reductant tubes and the sample probe from their respective beakers. With the pump roller continuing to turn, allow the tubes to dry for ~ 3-5 minutes.
 - 11.8.6. Click on the “Pump 1” button to stop the pump roller.
 - 11.8.7. Unscrew the tube coming from the autosampler Cell from the top of the Gas/Liquid Separator. Unscrew the cap from the Separator and dry the membrane. Replace the cap onto the Separator.
 - 11.8.8. Unlock the pressure adjustment levers from the tube magazines. Disconnect the tubes from the magazines to allow them to hang free.
 - 11.8.9. Shut all valves at the gas supply.
 - 11.8.10. Click “File,” then “Exit” to exit the AA Winlab software. The defaulted Desktop configuration will appear on the screen.
 - 11.8.11. Turn off the switch for the FIMS 100 System located on the front of the unit.
 - 11.8.12. Shut down the Dell computer as per Windows protocol. The system is now shut down.

12. ACCEPTABILITY OF QA DATA

- 12.1. Standard Curve Calibration- The calibration of the mercury standard curve is checked for linearity before samples are analyzed. The calibration curve must have a correlation coefficient of ≥ 0.995 . This value can be found on

the last page of the calibration data. If this criteria is not met, the problem must be found and corrected. The calibration procedure is then repeated.

- 12.2.** Quality Assurance Samples (matrix spikes, sample duplicates, LCS's, reagent blanks, and CRM's)- These QA parameters are tracked by the UC Davis Mercury Group and their results recorded onto control charts. Each chart is a graphical representation of the particular QA data, with upper and lower limits for data variance. The charts are used in determining if the analysis is "in control" and performing correctly according to analytical expectations. The procedure for developing control charts is found in *Standard Methods for the Examination of Water and Wastewater*, 18th Edition (1995). The data for each QA parameter is tracked and, when twenty data points have been accumulated, plotted on a control chart. Percent recovery or sample concentration values are used in the data plot. The mean (\bar{x}), standard deviation (s), upper and lower warning limits (UWL and LWL, $\pm 2s$), and upper and lower control limits (UCL and LCL, $\pm 3s$) are determined and used in construction of the chart. New control charts are prepared when twenty new data points are acquired. This allows the chart to be reflective of the twenty most recent data points. As analyses are performed, the QA results for each parameter are compared to the appropriate control chart. Interpretation of the control charts is as follows:

- 12.2.1.** If two consecutive QA results exceed a warning limit, the analysis is stopped immediately. The samples are reanalyzed. If the results are within the warning limits, the analysis is continued. If not, the source of the problem is determined before reanalysis or re-digestion.
- 12.2.2.** If one QA result exceeds a control limit, the analysis is stopped immediately. The sample is reanalyzed. If the result is within the control limits, the analysis is continued. If not, the source of the problem is determined before reanalysis or re-digestion.
- 12.2.3.** For sample duplicates, the UC Davis Mercury Group is currently using the commercial industry standard of 0-25% RPD (relative percent difference between duplicate analyses) for sample concentrations less than 20 times the MDL (<0.020 ug/g), and 0-15% RPD for sample concentrations greater than or equal to 20 times the MDL (≥ 0.020 ug/g).

- 12.3.** All control charts and accompanying data can be found in the UC Davis Mercury Group QA Manual located in the laboratory near the FIMS 100 System.

PERKIN-ELMER MANUALS FOR REFERENCE IN THE USE OF THE PERKIN-ELMER FIMS 100 SYSTEM

1. FIMS- FLOW INJECTION MERCURY SYSTEM, “INSTALLATION, MAINTENANCE, SYSTEM DESCRIPTION,” PUBLICATION # B3118.10, RELEASE 1.1 (JUNE 1994)
2. FIMS- FLOW INJECTION MERCURY SYSTEM, “SETTING UP AND PERFORMING ANALYSES,” PUBLICATION # B3118.20, RELEASE 1.1 (MARCH 1994)
3. FIMS- FLOW INJECTION MERCURY SYSTEM, “SOFTWARE GUIDE,” PUBLICATION # B3118.30, RELEASE 1.0 (JULY 1994)
4. FIMS- FLOW INJECTION MERCURY SYSTEM, “RECOMMENDED ANALYTICAL CONDITIONS AND GENERAL INFORMATION,” PUBLICATION # B3505.10, RELEASE 4.0
5. AS-90- AUTOSAMPLER FOR ATOMIC SPECTROSCOPY, “INSTALLATION, MAINTENANCE, AND SYSTEM DESCRIPTION,” PUBLICATION # B3109E, RELEASE 4.0 (APRIL 1994)

UC Davis SOP #4:

Analysis of Organic (Methyl) Mercury Using the Perkin-Elmer Flow Injection Mercury System (FIMS)

Last revised by Ron Weyand and Shaun Ayers
November 11, 2006

Originated by Shaun Ayers, Ron Weyand, and Darell Slotton

Dept. Of Environmental Science and Policy
University of California, Davis
One Shields Avenue
Davis, CA 95616

6. **SCOPE AND APPLICATION-** This SOP outlines sample extraction, digestion, and subsequent analysis of samples for determination of organic mercury in aquatic tissues. The analysis is performed by the Perkin-Elmer Flow Injection Mercury System, Model 100 (better known as the FIMS 100). The analysis method is based on EPA Method 245.6, as well as methodology developed within the UC Davis Mercury Group.
7. **METHOD DETECTION LIMITS-** MDL= 0.01 ug/g Hg (both wet and dry samples, 9/9/99 data)
8. **WORKING RANGE-** 0.01-3.0 ug/g (dry samples, 50mg sample size), 0.01-0.75 ug/g (wet samples, 200mg sample size)- (These ranges are based on a 1-ml sample loop. These ranges can be extended by use of smaller sample loops, allowing for analysis of high-level mercury samples).
9. **SUMMARY OF METHOD-** In order to be analyzed by the cold vapor atomic absorption technique, organic mercury needs to be extracted from the sample. Tissue samples of various aquatic species are shaken in a solution of copper sulfate/sodium bromide to convert the organic mercury (associated with protein sulfhydryl groups) to an organic Hg/bromide species. This species is then partitioned into 3:2 methylene chloride/hexane. An aliquot of this organic layer is mixed in a digestion tube with a nitric acid/sulfuric acid solution. The methylene chloride/hexane mixture is dissipated in a 95-degree C water bath, leaving the organic Hg/bromide species for further digestion (to the +2 oxidation state) in the nitric acid/sulfuric acid solution. This oxidation is followed by a second oxidation with a potassium permanganate/potassium persulfate solution. This oxidations is carried out in a 95-degree water bath. After reduction of any remaining permanganate by hydroxylamine hydrochloride, the samples are brought to volume in the reaction tubes with deionized water. The prepared samples are allowed to flow into the FIMS, along with an acidic carrier solution. The mercury in the sample digests is reduced to elemental mercury by reaction with stannous chloride in a reaction manifold. The gaseous elemental

mercury is stripped from the sample solution by argon gas in a gas/liquid separator. The mercury is swept by the gas into a long glass cell that is positioned along the light path of a high-intensity mercury lamp. The sample mercury in the cell absorbs some of the light emitted by the mercury lamp. This decrease in light energy is directly proportional to the amount of mercury in the sample. The FIMS 100 Spectrophotometer is an automated system operating under Microsoft Windows and offers many options for data calculation and reporting.

10. INTERFERENCES-

- 5.1. Inorganic Hg can also be released in the $\text{CuSO}_4/\text{NaBr}$ extraction step, but has been shown to reside exclusively in the aqueous phase.
- 5.2. Sulfur compounds in the sample, which are extracted into the methylene chloride/hexane phase, may complex with the organic mercury and cause low recoveries.
- 5.3. Some organic vapors, such as acetone and benzene, may interfere by absorbing at the 253.7 nm analysis wavelength. Use caution analyzing samples around these types of organic chemicals.
- 5.4. Any possible reduction interference from sulfide is eliminated by the addition of KmnO_4 . It has been shown that sulfide concentrations as high as 20 mg/L (S as Na_2S) do not interfere with mercury recovery from deionized water.
- 5.5. Samples high in chlorides may require additional KmnO_4 . Free chlorine is produced from chlorides in the sample during the oxidation step. This free chlorine has an absorption line near that of mercury (~253 nm). Addition of excess hydroxylamine hydrochloride to the sample, along with vigorous mixing and shaking of the sample, will purge free chlorine from the sample solution. The reaction tubes will be uncapped at the time of analysis, thus ensuring removal of contaminants from the dead-air space above the sample solutions.
- 5.6. Any interference from certain volatile organic materials (with absorption lines near 253 nm) will be removed when samples are shaken, than uncapped for analysis. This removes contaminants from the dead-air space above the sample.

6. SAFETY-

- 12.4. This analysis employs the use of various hazardous chemicals. All precautions should be taken to avoid exposure to these potentially dangerous chemicals. A lab coat and safety glasses are required at all times when performing this analysis. All digestions, preparation of reagents, and dissipation of waste mercury should be performed in the safety hood.
- 12.5. For specific information on the chemicals used in this analysis, please refer to the Material Safety Data Sheet (MSDS) for each specific chemical. The chemical MSDS will provide information on toxicity, flammability, handling protocol, and clean-up procedures in case of accidental spillage or contact.

13. INSTRUMENTATION/EQUIPMENT-

- 13.1. Perkin-Elmer FIMS 100 Spectrophotometer with the AS-90 Autosampler
- 13.2. Fisher Scientific 10-Liter Hot Water Bath
- 13.3. Ohaus "Precision Advanced" Model GT410 Top-Loading Analytical Balance
- 13.4. Thermolyne Cimarec 2 Hot Plate/Stirrer
- 13.5. Fisher Scientific "Genie 2" Vortex Mixer
- 13.6. Gilsop "Pipetman" Adjustable Pipetor
- 13.7. Eppendorf Repeater Pipetor
- 13.8. Kimble 20 ml glass culture tubes with caps
- 13.9. Biospec Products "Tissue Terror" homogenizer
- 13.10. Fisher Scientific "Centrifric" centrifuge
- 13.11. Various analytical glassware: volumetric flasks, graduated cylinders, beakers, etc.

14. REAGENTS AND STANDARDS-

- 14.1. Deionized Water (>17 umhos/cm)
- 14.2. Hexanes-use certified ACS grade or equivalent
- 14.3. Methylene Chloride-use certified ACS grade or equivalent
- 14.4. Cupric Sulfate-use certified ACS grade or equivalent
- 14.5. Sodium Bromide-use certified ACS grade crystals or equivalent
- 14.6. Stock Mercury Solutions- Prepare two separate stock solutions-one for the calibration curve and matrix spikes; the other for laboratory control samples (LCS's). Dry, certified mercuric chloride (HgCl_2) is used in stock solution preparation. Use different brands or lot numbers for the two stock solutions. Keep a log of all standard preparations in the UC Davis Mercury Group "Standard and Solution Preparation" notebook. These standard solutions will not be extracted (as they are inorganic Hg standards). Prepare as follows:
 - 14.6.1. Fill a 200 ml volumetric flask to ~50 ml with deionized water. Add 1.0 ml concentrated nitric acid (to acidify and preserve the solution) and mix. Weigh 0.2708g HgCl_2 onto clean weighing paper and transfer to the volumetric. Mix and bring to mark with deionized water to produce a 1000 ug/ml stock mercury standard. Repeat this procedure for the second stock solution. These solutions are stable for two years.
 - 14.6.2. Prepare a 10 ug/ml working mercury standard by half-filling a 100 ml volumetric with deionized water. Add 0.5 ml concentrated nitric acid and mix. Pipet 1.0 ml of the 1000 ug/ml mercury standard into the volumetric. Mix and bring to mark with deionized water to produce a 10 ug/ml mercury standard. Repeat the procedure for the second working solution. These solutions are stable for two months.
 - 14.6.3. Prepare a 0.100 ug/ml working mercury standard by half-filling a 100 ml volumetric flask with deionized water. Add 0.5 ml concentrated nitric acid and mix. Pipet 1.0 ml of the 10 ug/ml mercury standard into the volumetric. Mix and bring to mark with deionized water to produce a 0.100 ug/ml mercury standard. Repeat the procedure for the second

working solution. These solutions are stable for a period of two weeks after preparation. Be sure to keep solutions tightly closed.

- 14.7.** Concentrated Nitric Acid (HNO_3)- Use only Trace Metal Grade
- 14.8.** Concentrated Sulfuric Acid (H_2SO_4)-Use only Trace Metal Grade
- 14.9.** Concentrated Hydrochloric Acid (HCl)- Use only Trace Metal Grade
- 14.10.** Potassium Permanganate (KmnO_4)- Use only dry, certified reagent labeled “suitable for mercury determination.”
- 14.11.** Potassium Persulfate ($\text{K}_2\text{S}_2\text{O}_8$)-Use only dry, certified reagent labeled “suitable for mercury determination.”
- 14.12.** Sodium Chloride (NaCl)-Use ACS Reagent Grade or equivalent.
- 14.13.** Hydroxylamine Hydrochloride ($\text{NH}_2\text{OH}\cdot\text{HCl}$)-Use only reagent labeled “suitable for mercury determination.”
- 14.14.** Sodium Hydroxide (NaOH) pellets- Use any grade.
- 14.15.** Bromocresol Green/Methyl Red indicator solution- Dissolve 0.1g bromocresol green (sodium salt) and 0.1g methyl red (sodium salt) in 100 ml DI water. One can also use a commercially-prepared solution of these two chemicals.
- 14.16.** Sulfuric Acid (4N)- Add 111 ml concentrated sulfuric acid to about 750 ml DI water. Swirl to mix and allow to cool. Bring volume to 1 liter with DI water. Make fresh daily.
- 14.17.** Cupric Sulfate solution (2.5 w/v)-Dissolve 5.0g cupric sulfate ($\text{CuSO}_4\cdot 5\text{H}_2\text{O}$) in 150 ml 4N sulfuric acid. Swirl to mix and bring volume to 200 ml with DI water. Make fresh daily.
- 14.18.** Sodium Bromide solution (30% w/v)-Dissolve 60g sodium bromide (NaBr) in 150 ml 4N sulfuric acid. Swirl to mix and bring volume to 200 ml with DI water. Make fresh daily.
- 14.19.** Methylene Chloride/Hexanes solution (3:2)-Mix together methylene chloride and hexanes using a 3:2 ratio of volumes.
- 14.20.** Potassium Permanganate/Potassium Persulfate oxidizing solution (5%/5% w/v)- Fill a 1-liter volumetric flask to ~ 700 ml with deionized water (do not use this volumetric flask for any other reagent preparation). Add 50g potassium permanganate and 50g potassium persulfate to the flask. Swirl to mix. Gently warm and stir the solution on a hot plate/stirrer to help dissolve the reagent crystals. When dissolved, remove from the plate, cool, and bring to mark with deionized water. This solution is stable for one year.
- 14.21.** Sodium Chloride/Hydroxylamine Hydrochloride clearing solution (12%/12% w/v)- Weigh 6g sodium chloride and 6g hydroxylamine hydrochloride and add to a 50 ml volumetric flask. Add a few mls of deionized water and swirl to mix. When crystals are dissolved, bring to mark with deionized water. This solution must be prepared fresh on the day of use.
- 14.22.** 3% Hydrochloric Acid carrier solution- Fill a 2-liter volumetric flask with ~1500 ml of deionized water. Add 60 ml concentrated hydrochloric acid while swirling. Add deionized water and bring to mark. Prepare this reagent fresh on the day of use.

- 14.23.** 1.1% w/v Stannous Chloride reducing solution- Fill a 1-liter volumetric flask with ~ 500 ml deionized water. Add 30 ml concentrated hydrochloric acid while swirling. Weigh and add 11g stannous chloride ($\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$) to the flask and swirl to dissolve the crystals. When dissolved, bring to mark with deionized water. This solution must be prepared fresh on the day of use. Do not use any glassware or utensils that have come into contact with stannous chloride in preparation of other reagents. (Caution: Historically, it has been found that certain package lots of stannous chloride do not allow full recovery of mercury from certified reference materials (CRM's) and samples. Be sure to test each lot of stannous chloride by analyzing replicate samples of the CRMs that are used in the laboratory. If recoveries are not acceptable, do not use the problematic stannous chloride in mercury determinations.)
- 14.24.** 2:1 Sulfuric Acid/Nitric Acid digestion solution- Place one part concentrated nitric acid into an appropriately-sized beaker. While swirling, add two parts concentrated sulfuric acid to the nitric acid in the beaker (caution- this procedure produces a vigorous exothermic reaction). Prepare this solution fresh on the day of use.
- 14.25.** Sodium Hydroxide solution for waste trap- During the course of the analysis of samples, the Hg standards, samples, and reaction chemicals are collected in a large plastic container. This mixture is acidic due to the 3% HCl solutions and the sample/standard digests. Sodium Hydroxide is used to neutralize and destroy this acidic mixture during the analytical run. Any Hg from the digests is also removed in this container due to the reaction with stannous chloride. The Hg is removed from solution and is trapped on a carbon filter. This neutralized analysis waste can then be discarded.
- 14.25.1.** Assemble the waste trap as depicted in Appendix 3.
- 14.25.2.** Remove the rubber stopper. Fill the plastic container with ~ 1 liter of water.
- 14.25.3.** Using equivalent weights of HCl, HNO_3 , and H_2SO_4 , calculate the equivalent weight of NaOH needed to neutralize all sample and reagent solutions. Weigh the NaOH and add to the container of water. Swirl to dissolve.
- 14.25.4.** Place the container on the stir plate and begin stirring the solution. Add a few drops of the bromocresol green/methyl red indicator solution. The solution will now be blue/green in appearance.
- 14.25.5.** Replace the stopper, making sure that the container is airtight. Turn on the laboratory air valve in order to produce bubbling in the waste trap. Adjust the bubble rate to ~ 2 bubbles/second. The waste trap is now ready for use.
- 14.25.6.** During the course of the analysis, monitor the color of the waste solution in the trap. If it begins to turn red, add more NaOH pellets until the solution is once again blue/green. Check the pH of the solution at the end of the analysis with pH paper. Adjust the pH of the solution with NaOH pellets or dilute HCl as needed to produce a neutral-pH solution. Once the solution is at neutral pH, it can be discarded.

15. SAMPLE EXTRACTION AND DIGESTION (always performed in a fume hood)-

- 15.1. Prepare an Analysis Worksheet containing all pertinent sample information. This worksheet is prepared by computer under the Microsoft Excel format. A copy of the full printout of the analysis worksheet is attached at the end of this SOP as Appendix 1. The full worksheet is normally not printed and used throughout the analysis. Only data such as culture tube numbers, sample weights, and full post-digested tube weights are recorded on a partial hard-copy of the analytical worksheet. This copy of the worksheet accompanies the samples through the digestion and analytical procedures. All data, including that on the hardcopy, is transferred to the analytical worksheet in the computer, thus allowing the computer to calculate analytical parameters and results.
- 15.2. Set up the appropriate number of clean 20-ml culture tubes in one or more tube racks for the extraction, as well as the digestion.
- 15.3. Number the tubes with a permanent black marking pen. Also, number the culture tube caps with the corresponding number using an electric engraver.
- 15.4. Weigh each empty tube (including the cap) and record the weight on the worksheet. Alternatively, the tubes may be weighed in advance and the weights entered into a computer database. The database can then be accessed at the time of analysis to obtain the needed tube weights.
- 15.5. Turn on the hot water bath and set the "Temperature Set" knob to $\sim 95^\circ$. Allow the bath to heat up while preparing the samples for extraction and digestion. Once the bath has reached temperature, use a certified thermometer to measure the bath water temperature. Adjust the "Temperature Set" knob as necessary to produce a water bath temperature of $95 \pm 1.0^\circ\text{C}$. Record the thermometer temperature and the digital readout from the water bath in the "UC Davis Mercury Group Equipment Manual."
- 15.6. Add samples, standards, and Quality Assurance (QA) samples to the appropriate culture tubes as follows:
 - 15.6.1. For dry samples and Certified Reference Materials (CRM's) to be extracted, place a culture tube (without cap) on the Ohaus analytical balance and tare its weight. Mix the sample in its container by rolling and/or shaking. Place ~ 50 mg of dry sample into the culture tube and replace on the balance. Record the sample weight on the worksheet. Cap and place the tube back into the rack. Place a corresponding digestion sample tube for each extraction tube into a tube rack for later digestion.
 - 15.6.2. For the standard curve, pipet the appropriate amount of standard directly into the digestion culture tube (remember-these standards are not extracted). The standard mercury amounts currently used for the standard curve are as follows:

<u>Standard #</u>	<u>Hg Conc.</u>	<u>Mls 0.100 ug/ml Hg Std added</u>
1	0 ng	0
2	10 ng	0.10
3	20 ng	0.20
4	40 ng	0.40
5	60 ng	0.60
6	90 ng	0.90
7	120 ng	1.20
8	150 ng	1.50

15.6.3. In order to assess the accuracy and precision of the analytical method, different types of Quality Assurance (QA) samples are extracted and/or digested, then analyzed along with the samples. The type of QA samples analyzed and their frequencies are as follows:

- 15.6.3.1. Matrix Spike-** A matrix spike is analyzed once every ten samples. The matrix spike is prepared by adding a known amount of mercury to the sample matrix being analyzed. Currently, a known amount of organic mercury is added to the matrix to prepare the matrix spike. Certified Reference Material (CRM-see below) is used in matrix spike preparation. These materials have certified values for organic mercury. The unit for the matrix spike level is ug/g.
- 15.6.3.2. Sample Duplicate or Split-** A sample duplicate or split is analyzed once every ten samples. The duplicate is an “extra” analysis of the same sample. The duplicate analysis is performed on the same sample used for the matrix spike preparation.
- 15.6.3.3. Laboratory Control Standard (LCS)-** An LCS is analyzed once every twenty samples and is not extracted. The LCS is prepared by adding a known amount of mercury to deionized water in a digestion tube, which is then carried through the entire digestion and analytical procedure. The source of mercury for the LCS must be of a different lot number or manufacturer than that of the matrix spike mercury source. Most LCS concentrations are within the range of 10-50ng. The level is reported as ug/L.
- 15.6.3.4. Reagent Blank-** A reagent blank is analyzed once every twenty samples. The reagent blank is prepared by using deionized water in place of sample matrix, then carried through the entire extraction and digestion procedure. The units of reporting are ug/L.
- 15.6.3.5. Certified Reference Material (CRM)-** Different CRM types, in duplicate, are analyzed per analytical run. Examples of the types of CRMs used are the TORT-2 (lobster hepatopancreas) and DOLT-2 (dogfish liver) materials furnished by the National Research Council of Canada, and NIST 2976 Mussel tissue furnished by the National Institute of Standards and Technology.

Other CRM's may be available for use. These are sample matrixes that have been characterized and certified for organic mercury content. Each CRM is carried through the extraction and digestion procedure and the percent recoveries of the analyte mercury are determined.

- 15.7.** Place all samples that will be extracted for organic mercury into the fume hood. Using a graduated pipette, dispense 6 mls of the cupric sulfate solution into each extraction tube.
- 15.8.** Add 4.5 mls of the sodium bromide solution to each extraction tube using a graduated pipette.
- 15.9.** Mix each sample with the cupric sulfate/ sodium bromide solution by placing the end of the Biospec Products "Tissue Terror" homogenizer into the sample tube, turning it on, adjusting the homogenizing speed to maximum, and homogenizing for a few seconds. Homogenizing time can be adjusted upward if the sample requires more time for complete homogenization. Lift the end of the homogenizer out of the solution and, while continuing to run, lightly tap the sides of the extraction tube with the end of the homogenizer to dislodge any sample that may have adhered to the homogenizer. When homogenizing is complete, remove the homogenizer from the extraction tube and rinse the end into a waste beaker with DI water to clean it.
- 15.10.** Cap each sample tube and shake for 1 minute to ensure complete mixing.
- 15.11.** Remove cap from each sample tube and add 4.0 mls of the methylene chloride/hexane solution to each tube using a volumetric pipette.
- 15.12.** Cap each tube after addition of the organics and shake once again for 1 minute to perform the extraction. At the completion of the shake, organic mercury will be in the methylene chloride/hexane layer.
- 15.13.** Place each tube into the centrifuge and centrifuge the samples at a setting and time to ensure any particles suspended in the organic area are removed from the organic layer.
- 15.14.** Add 1.0 ml of the 2:1 sulfuric acid/nitric acid digestion solution to each digestion tube. Using the "Pipetman" pipettor, place 3.0 mls of the organic layer of each tube into its corresponding digestion tube by drawing and dispensing 3-1.0 ml portions. Vortex the digestion tube to ensure mixing between the acid and organic layers.
- 15.15.** Place the digestion tubes containing the organic and acid layers into an open 95-degree water bath and allow the organic layer to dissipate until no organic layer remains and the "organic fog" is no longer present above the water bath.
- 15.16.** Remove the samples from the water bath and cap.
- 15.17.** Place sample rack(s) containing all tubes (including standards and LCS's that were not extracted) into the 95°C water bath. Place the water bath cover over the water bath and digest the sample tubes for one hour. Begin the timing of the digestion when the water bath returns to 95°C.

- 15.18.** While the samples are digesting, warm and stir the potassium permanganate/potassium persulfate oxidizing solution (see 8.10.) on a hot plate/stirrer to re-dissolve any crystals that may have formed upon storage.
- 15.19.** After one hour of digestion, carefully remove the cover from the water bath (avoid dripping any water onto the samples). Remove the samples from the water bath and allow to cool. Replace the cover on the water bath to maintain its 95°C temperature.
- 15.20.** When cool, uncapped the sample tubes (the contents may be under pressure-use gloves and caution). Set the caps aside. Pipet one ml of the warmed-and-stirred potassium permanganate/potassium persulfate oxidizing solution into each tube (this may produce a reaction in the tube, so add with caution). Stir or vortex the samples to mix. Add another one-ml aliquot of the oxidizing solution to each tube (for a total of two ml of oxidizing solution per tube). Stir or vortex each tube to ensure complete mixing.
- 15.21.** Place the uncapped tubes back into the 95°C water bath and carefully, without dripping, place the cover on the water bath. Allow the samples to digest for two hours. Begin timing of the digestion when the water bath returns to 95°C. Periodically check the samples to ensure that the potassium permanganate does not change to a ruddy brown color. This would suggest a sample high in organic content. Potassium permanganate must be kept in excess throughout the digestion process. If necessary, add an additional 0.5 ml of the oxidizing solution until the color of the solution remains the purple permanganate color.
- 15.22.** After two hours of digestion, carefully remove the cover from the water bath (avoiding any water dripping into the samples). Remove the samples from the water bath and allow to cool. The samples can now be capped and stored for no more than two days, or the samples can be prepared for analysis as follows:
- 15.23.** If samples have been stored, remove the caps and put them aside. Add 0.5 ml of the sodium chloride/hydroxylamine hydrochloride clearing solution to each tube. Swirl or vortex to mix. This neutralizes any excess potassium permanganate present in the tube. The sample solutions should become colorless and, depending on the sample matrix, reasonably clear.
- 15.24.** Fill each tube with fresh deionized water up to the level demarcated by the top of each culture tube white label. This corresponds to approximately 16-17 ml total sample volume.
- 15.25.** Cap each tube and shake the tubes to ensure proper mixing of the deionized water and digested-sample liquid (if mixing is not done properly, inconsistent sample results will occur upon analysis).
- 15.26.** Make sure each tube is clean and dry by wiping with a clean tissue or Kimwipe.
- 15.27.** Weigh each clean, dry sample tube on the top-loading balance. Record each weight on the analysis worksheet. Determine the weight (and, subsequently, the volume in mls) of each sample by subtracting the empty tube-and-cap weight from the full tube-and-cap weight (this is automatically done by

computer upon transferring all data to the analysis worksheet in the computer). The samples are now ready for analysis.

16. SET-UP OF THE PERKIN-ELMER FIMS 100 ANALYTICAL SYSTEM FOR SAMPLE ANALYSIS (for further information, consult the various manuals supplied by Perkin-Elmer regarding all parameters of the FIMS analytical systems. These are listed at the end of this SOP as Appendix 2)

- 16.1.** At least one hour before analysis, turn on the FIMS system by depressing the “on” switch on the front of the Dell computer, as well as depressing the “on-off” switch on the front of the FIMS 100 unit, which is set below the AS-90 Autosampler. This allows the mercury lamp within the FIMS unit to warm and stabilize before analysis.
- 16.2.** Open the gas cylinder main valve (on top of the cylinder) and the gas outlet valve (small black knob located on the left side of the gas valve assembly). Use the large black valve located on the front of the gas valve assembly to adjust the gas pressure to 360 kPa.
- 16.3.** Upon start-up of the computer, Windows will initiate the operating system.
- 16.4.** Click “Cancel” when the “Enter Network Password” window appears.
- 16.5.** Click “Exit” when the “Program Diskettes” window appears. This bring the screen to the default Desktop configuration.
- 16.6.** Double-click on the “AA Winlab Analyst” icon to initiate the Perkin-Elmer AA Winlab, V. 2.50, Instrument Control software.
- 16.7.** Select a workspace by clicking on the “Use a custom-designed workspace” icon, then choose the file name by selecting the “sma.fms” file. Click on “OK” to open the workspace. The screen will then display all the pertinent windows used in the analytical procedure. The windows displayed are as follows:
 - 16.7.1.** Automated Analysis
 - 16.7.2.** Examine Peak
 - 16.7.3.** FIAS Control
 - 16.7.4.** Examine Calibration
 - 16.7.5.** Results
 - 16.7.6.** AA Winlab toolbar
- 16.8.** Click “File,” highlight “New” with the cursor, then click “Sample Info File.” This displays the Sample Information Editor window. This window is used to enter and save sample information into a file used by the FIMS system.
 - 16.8.1.** Enter the description of the analytical set (Example: 11Delta99, 01Sed99, etc.).
 - 16.8.2.** Enter the batch ID, if necessary (this parameter is usually left blank).
 - 16.8.3.** Enter the initials of the analyst.
 - 16.8.4.** Enter the sample volume as “16.000.” This is the approximate volume of the samples.

- 16.8.5.** Click on the arrow next to “Volume Units” and choose “ml.” This is the unit of volume used for the samples.
 - 16.8.6.** Click on the arrow next to “Weight Units” and choose “g.” This is the unit of weight used for the samples.
 - 16.8.7.** Sample descriptions will now be entered in the “Sample ID” column. Click on the “Sample ID” box corresponding to the autosampler location for the first sample (not calibration standard). Use the scroll bar on the far right side to move to the required location. Currently, most samples analyzed by the UC Davis Mercury Group begin at autosampler location “17.” Enter the sample description.
 - 16.8.8.** Tab right to the “Sample Weight” column. Enter the sample weight in grams.
 - 16.8.9.** Fill in the “Sample ID” and “Sample Weight” columns for all the samples.
 - 16.8.10.** Double-click on the gray box labeled “Sample Units.” The “Sample Units Column Fill” window will appear. Select the units that the computer will use to report the final sample results by clicking on the small circle next to “Weight/Weight Units.” Click on “ug/g.”
 - 16.8.11.** Move to the Location Range area and select the autosampler location for the first sample of the analytical run (Start), then select the autosampler location for the last sample of the analytical run (End). Click “OK.” The units of “ug/g” should appear in the “Sample Units” column of each sample.
 - 16.8.12.** The final sample volume for each sample is not exactly 16.000 mls, as was entered in 10.8.4., above. Due to this fact, a dilution factor must be entered into the computer to be used by the computer in correctly calculating the final result. This factor for each sample will be entered in the “User Dilution” column. Calculate the dilution factor for each sample by taking its final weight or volume (see 9.19.) and dividing by “16.” This number, usually over 1.0, is entered for each sample in the “User Dilution” column.
 - 16.8.13.** Enter any necessary remarks for each sample in the “Remarks” column.
 - 16.8.14.** Click on “File,” then click on “Save.” The “Save As” window appears. Give the sample batch, that was just entered, a file name. The file name cannot be more than eight characters in length. After entering the file name, click “OK.” The file is now saved in the computer software.
 - 16.8.15.** Close the Sample Information Editor window.
- 16.9.** Choose an analytical method for the samples by double-clicking on the box directly below the “Method” title in the “Setup” tab of the Automated Analysis window. This displays the “Open Method” window. Highlight the method required for analysis in the “Name” column. For most samples, use

the “1 ml method” (if samples are high in mercury content, use of a 0.5 ml sample loop and corresponding “0.5 ml method” may be required). Use the scroll bar on the far right of the window, if necessary, to move to the appropriate method. Click “OK” to select the highlighted method. The Automated Analysis window will now display the selected method in the “Method” column.

- 16.10.** Click on the “MethEd” icon in the toolbar. The “Method Editor” window for the selected method is displayed. This window allows the analyst to modify certain parameters regarding the selected method. There are six different subgroups (shown as tabs along the bottom of the window) within the window that show various areas of modification. The subgroups and the required settings for the various parameters within each subgroup are as follows:

- 16.10.1. Inst.-** These are important instrument settings for the selected method. The normal settings for the “1 ml method” are as follows:

- 16.10.1.1. Method Desc.-** 1 ml injection 0-250 ng Hg
- 16.10.1.2. Wavelength-** 253.7
- 16.10.1.3. Read Time (sec)-** 25.00
- 16.10.1.4. Read Delay (sec)-** 0.0
- 16.10.1.5. BOC Time (sec)-** 2
- 16.10.1.6. Type-** Atomic Absorption
- 16.10.1.7. Measurement-** Peak Height
- 16.10.1.8. Smoothing (pts)-** 9

- 16.10.2. Calib.-** This tab has three pages of calibration parameters that can be modified. The settings for the “1 ml method” are as follows:

- 10.10.2.1. Equation , Units, & Replicates**

- 16.10.2.1.1. Equation-** Zero Intercept: Linear
- 16.10.2.1.2. Max. Decimal Places-** 3
- 16.10.2.1.3. Max. Significant Figures-** 4
- 16.10.2.1.4. Calibration-** ug/L
- 16.10.2.1.5. Sample-** ug/L
- 16.10.2.1.6. Replicates-** Choose the “fixed” option and set to “2.” Click on the “Set” button. In the “Samples” field, select the first option and set the replicate number to “1.” In the “Calibration Solutions” field, set the replicate number to “1.” In the “QC Samples” field, select the first option. When complete with all fields, click “OK.”

16.10.2.2. Standard Concs (page name)- This page is used to input the standard curve identifications, concentrations, and autosampler locations.

16.10.2.2.1. The “ID” and “A/S Loc.” (autosampler location) columns will contain the following identifications: Calib. Blank = Calib Blank, A/S loc. = 9; Reslope Std. = Reslope, A/S loc. = (not used); Reagent Blank = Reagent Blank, A/S loc. = (not used); Standard 1 = 10 NG, A/S loc. = 10; Standard 2 = 20 NG, A/S loc. = 11; Standard 3 = 40 NG, A/S loc. = 12; Standard 4 = 60 NG, A/S loc. = 13; Standard 5 = 90 NG, A/S loc. = 14; Standard 6 = 120 NG, A/S loc. = 15; Standard 7 = 150 NG, A/S loc. = 16

16.10.2.2.2. The “Conc.” Column changes for each analytical run. The standard concentrations in ug/L are entered here. To determine these values, divide the standard concentrations in ng by their appropriate final volumes (see 9.19.). The resulting numbers are the standard concentrations in ug/L. Enter the value for each standard in its appropriate box.

16.10.2.3. Auto-Sampler (page name)-

16.10.2.3.1. Tray Type- Tray B

16.10.2.3.2. Frequency- Between Samples

16.10.2.3.3. $\sqrt{\quad}$ - Wash only if sample “Concentration” > “3” ug/L

16.10.2.3.4. Location of Wash Solution- 0

16.10.2.3.5. Cycles: Wash for “1” cycles

16.10.2.3.6. Use FIAS steps “1” to “1” as programmed

16.10.3. FIAS- This tab lists important pump and valve parameters that can be edited. Settings for the “1 ml method” are as follows:

16.10.3.1. Sample Volume (ul)- 1000

16.10.3.2. Use Amalgam- No

16.10.3.3. The “Flow Injection Program” columns should be set as follows: Prefill-Time (sec) = 10, Pump 1 Speed = 120, Valve = Fill; 1- Time (sec) = 15, Pump 1 Speed = 120,

Valve = Fill; 2- Time (sec) = 25, Pump 1 Speed = 120,
Valve = Inject, highlight “read step.”

16.10.3.4. Steps to Repeat- “1” to “1.”

16.10.3.5. Number of Repeats- 0

16.10.4. Checks- This tab lists various checks on calibration and analysis measurements that can be edited. Settings for the “1 ml method” should all be highlighted “No.”

16.10.5. QC- This tab lists various QC calculations and procedures that can be performed by the computer. It contains four different pages. The UC Davis Mercury Group does not use this tab. Keep all fields blank or in default settings to disregard.

16.10.6. Options- This tab lists various optional parameters that can be edited. The settings for the “1 ml method” are as follows:

16.10.6.1. The “Results Display/Printed Log” field should be set to “Default.”

16.10.6.2. The “Peaks to Save” field should be set to “None.”

16.10.7. When all fields have been set, click “File,” then “Save.” All parameters are saved for the “1 ml method.” Close the “Method Editor” window.

16.11. Move to the Automated Analysis window and click “Browse” next to the field labeled “Sample Information File.” The File List window will appear. Highlight the analysis file that was produced in 10.8.14. above, then click “OK.” This will set up the FIMS system to analyze the selected analytical set. The file name should now appear in the “Sample Information File” field.

16.12. In the same Automated Analysis window, click “Browse” next to the field labeled “Results Data Set Name.” The “Choose Results Data Set” window will appear. Type in the result name for the analytical set in the “Result Name” field. This will be name for the sample results for this analytical set (it is usually given the same name as the Sample Information File). Click “OK” to save this name. The name should now appear in the “Results Data Set Name” field.

16.13. In the Automated Analysis window, move to the “During Analysis” field and highlight “Save Data” and “Print Log.” In the “Off After Analysis” field, highlight “Pump.”

16.14. At the bottom of the Automated Analysis window, click the “Analyze” tab to display the buttons and fields necessary to start/stop the analysis.

16.15. Move to the “FIAS Control” window and the “Manual Operation” field. Make sure the “Valve Fill/Inject” settings for Pumps 1 and 2 are set to “120.”

16.16. The computer and software parameters required for automated sample analysis are now set.

16.17. Install the pump tubing on the pump magazines as follows:

- 16.17.1.** An illustration as to the different types of pump tubing and their installation locations can be found in the manuals listed at the end of this SOP as Appendix 2. All tubing and valve openings must be free of any obstructions. Flush obstructed valve openings with pressurized deionized water and replace any tubing that is blocked.
 - 16.17.2.** Remove the pump magazines from their prospective pivots. The pump tubing can be installed with the magazines in place, but the tubing is easier to install without being attached to the pivots.
 - 16.17.3.** Gently pull the tubing and hook the tensioned tubing in the appropriate pump magazine between two of the collars on the pump tube.
 - 16.17.4.** Slide each pump magazine onto the appropriate pivot above or below the pump. Remember that solution flow is from left to right for pump magazines placed above the pump roller, and right to left for pump magazines placed below the pump roller.
 - 16.17.5.** Turn the pressure adjustment lever until the end of the pressure screw locks into the notch at the back of the pump magazine.
- 16.18.** Be sure that all tubing connections are secure and tight on both the FIAS Valve and Gas-Liquid Separator. If any tubing is damaged or kinked, replace with new tubing identical in part number to the original tubing. For the “1 ml method,” install the proper tubing as follows:
- 16.18.1.** Install the 1-ml sample loop on the FIAS Valve. Be sure that both ends are installed tightly.
 - 16.18.2.** Install tube # 198097 to the two manifold blocks of the Gas/Liquid Separator. Be sure that both ends do not kink and that they are installed tightly.
 - 16.18.3.** Install a 500 ul sample loop between the center manifold block and the main Gas/Liquid Separator block. This is the chemical reaction coil. Be sure that both ends do not kink and that they are installed tightly.
- 16.19.** Place the ends of the carrier and reductant tubes into separate beakers filled with deionized water. Place the ends of the sample and carrier waste tubes into a large plastic waste container.
- 16.20.** Fill the plastic wash beaker, located within the AS-90 Autosampler sample tray, with deionized water and place below the sample probe on the autosampler. Click on the “Move Probe Up/Down” button on the Automated Analysis window. This will move the sample probe down into the wash beaker filled with deionized water.
- 16.21.** Click on the “Pump 1” button in the “Manual Operation” field of the “FIAS Control” window. This will initiate the pump roller to turn. Deionized water should flow through the carrier, reductant, and sample probe tubes.
- 16.22.** After initiation of the pump roller, check the flow rates of the various tubes as follows:

- 17.3. Place the filled autosampler tray on top of the AS-90 Autosampler. Be sure the tabs on the bottom corners of the tray “lock” into place on top of the AS-90. This keeps the tray from moving during analysis.
- 17.4. Move to the Automated Analysis window on the computer screen and click on the “Calibrate” button. The FIMS System will automatically initiate the calibration procedure and begin analyzing the mercury standards for the calibration curve. The printer will print the calibration data as the procedure continues. As the calibration proceeds, monitor the various windows on the computer screen. If any data looks unacceptable, re-click on the “Calibrate” button when the autosampler is between samples. This action will stop the analysis. Correct any suspected problems, then re-click on the “Calibrate” button. This will once again initiate the calibration procedure.
- 17.5. When the calibration procedure is completed, highlight the “Examine Calibration” window. Click on “File,” highlight “Print,” then click on “Window Image.” This will tell the printer to print the calibration plot before analysis of the samples occurs.
- 17.6. After examination and acceptance of the calibration data, click the “Analyze Samples” button in the Automated Analysis window. This will automatically initiate the sample analysis mode of the FIMS System. As with the calibration procedure, monitor the computer screen for any anomalies in the sample analysis. If the sample analysis needs to be terminated or interrupted, click on the “Analyze Samples” button. This will interrupt the analysis. When the analysis is set to continue, re-click the “Analyze Samples” button. The “Continuing an Analytical Sequence” window will appear. Select the appropriate selection, then click “OK.” This will once again initiate the sample analysis (depending on the parameters chosen in the “Continuing an Analytical Sequence” window).
- 17.7. As the sample analysis continues, the printer will print sample results expressed in both “ug/g” and “ug/L” units. These results can then be inputted to the appropriate reporting format required for expression of results. The “ug/g” and “ug/L” results must be multiplied by the factor of 1.333 to account for the fact that only 3.0 mls of the methylene chloride/hexane layer was analyzed from the original 4.0 mls of the initial extraction.
- 17.8. Upon completion of sample analysis, the AS-90 Autosampler will move the sample probe to a location above the wash beaker. The pump roller will stop. The printer will print the results for the last samples. Shut down the FIMS 100 System by the following:
 - 17.8.1. Fill the wash beaker with deionized water and click on “Move Probe Up/Down” to lower the sample probe into the water.
 - 17.8.2. Fill two beakers with deionized water and place the ends of the Carrier and Reductant tubes into the water.
 - 17.8.3. Click the “Pump 1” button in the “Manual Operation” field of the “FIAS Control” window. This will initiate the pump roller and

deionized water will flow through all of the tubes. Let the tubes rinse for ~ 5-10 minutes.

- 17.8.4. While the tubes rinse, remove the rubber stopper from the waste trap plastic container. While the waste solution continues to stir, pour all standard and sample digest solutions into the container. Also, pour the SnCl_2 solution into the container. This will destroy and neutralize all acid and mercury from these solutions. Replace the rubber stopper.
- 17.8.5. Remove the ends of the Carrier and Reductant tubes and the sample probe from their respective beakers. With the pump roller continuing to turn, allow the tubes to dry for ~ 3-5 minutes.
- 17.8.6. Click on the "Pump 1" button to stop the pump roller.
- 17.8.7. Unscrew the tube coming from the autosampler Cell from the top of the Gas/Liquid Separator. Unscrew the cap from the Separator and dry the membrane. Replace the cap onto the Separator.
- 17.8.8. Unlock the pressure adjustment levers from the tube magazines. Disconnect the tubes from the magazines to allow them to hang free.
- 17.8.9. Shut all valves at the gas supply.
- 17.8.10. Click "File," then "Exit" to exit the AA Winlab software. The defaulted Desktop configuration will appear on the screen.
- 17.8.11. Turn off the switch for the FIMS 100 System located on the front of the unit.
- 17.8.12. Shut down the Dell computer as per Windows protocol. The system is now shut down.

18. ACCEPTABILITY OF QA DATA

- 18.1. Standard Curve Calibration- The calibration of the mercury standard curve is checked for linearity before samples are analyzed. The calibration curve must have a correlation coefficient of ≥ 0.995 . This value can be found on the last page of the calibration data. If this criteria is not met, the problem must be found and corrected. The calibration procedure is then repeated.
- 18.2. Quality Assurance Samples (matrix spikes, sample duplicates, LCS's, reagent blanks, and CRM's)- These QA parameters are tracked by the UC Davis Mercury Group and their results recorded onto control charts. Each chart is a graphical representation of the particular QA data, with upper and lower limits for data variance. The charts are used in determining if the analysis is "in control" and performing correctly according to analytical expectations. The procedure for developing control charts is found in *Standard Methods for the Examination of Water and Wastewater*, 18th Edition (1995). The data for each QA parameter is tracked and, when twenty data points have been accumulated, plotted on a control chart. Percent recovery or sample concentration values are used in the data plot. The mean (\bar{x}), standard deviation (s), upper and lower warning limits (UWL and LWL, $\pm 2s$), and upper and lower control limits (UCL and LCL, $\pm 3s$) are determined

and used in construction of the chart. New control charts are prepared when twenty new data points are acquired. This allows the chart to be reflective of the twenty most recent data points. As analyses are performed, the QA results for each parameter are compared to the appropriate control chart. Interpretation of the control charts is as follows:

- 18.2.1.** If two consecutive QA results exceed a warning limit, the analysis is stopped immediately. The samples are reanalyzed. If the results are within the warning limits, the analysis is continued. If not, the source of the problem is determined before reanalysis or re-digestion.
 - 18.2.2.** If one QA result exceeds a control limit, the analysis is stopped immediately. The sample is reanalyzed. If the result is within the control limits, the analysis is continued. If not, the source of the problem is determined before reanalysis or re-digestion.
 - 18.2.3.** For sample duplicates, the UC Davis Mercury Group is currently using the commercial industry standard of 0-25% RPD (relative percent difference between duplicate analyses) for sample concentrations less than 20 times the MDL (<0.20 ug/g), and 0-10% RPD for sample concentrations greater than or equal to 20 times the MDL (≥0.20 ug/g).
- 12.3.** All control charts and accompanying data can be found in the UC Davis Mercury Group QA Manual located in the laboratory near the FIMS 100 System.

PERKIN-ELMER MANUALS FOR REFERENCE IN THE USE OF THE PERKIN-ELMER FIMS 100 SYSTEM

1. FIMS- FLOW INJECTION MERCURY SYSTEM, “INSTALLATION, MAINTENANCE, SYSTEM DESCRIPTION,” PUBLICATION # B3118.10, RELEASE 1.1 (JUNE 1994)
2. FIMS- FLOW INJECTION MERCURY SYSTEM, “SETTING UP AND PERFORMING ANALYSES,” PUBLICATION # B3118.20, RELEASE 1.1 (MARCH 1994)
3. FIMS- FLOW INJECTION MERCURY SYSTEM, “SOFTWARE GUIDE,” PUBLICATION # B3118.30, RELEASE 1.0 (JULY 1994)
4. FIMS- FLOW INJECTION MERCURY SYSTEM, “RECOMMENDED ANALYTICAL CONDITIONS AND GENERAL INFORMATION,” PUBLICATION # B3505.10, RELEASE 4.0
5. AS-90- AUTOSAMPLER FOR ATOMIC SPECTROSCOPY, “INSTALLATION, MAINTENANCE, AND SYSTEM DESCRIPTION,” PUBLICATION # B3109E, RELEASE 4.0 (APRIL 1994)

UC Davis SOP #5: Stable Isotope Analysis for plant and animal tissue

Last revised by Darell Slotton
April 22, 2004

Originated by Amanda L. Bern

UC-Davis Clear Lake Environmental Research Center 200 Park St. Lakeport, Ca 95453

1. SCOPE AND APPLICATION

This SOP details the procedure for **laboratory stable isotope analysis of carbon, nitrogen and sulfur for plant and animal tissue.**

2. SUMMARY OF METHOD

Samples are dried at 55-75 °C in a laboratory drying oven or until a constant weight is achieved. Larger samples are ground in a ball mill for approximately 2 hours or until the material is a fine powder. Smaller samples are ground by hand with a porcelain mortar and pestle or with an electric grinder into a fine powder. Approximately 2 mg dried and ground material is weighed into a small tin cup for C and N analysis. Approximately 10 mg of dried and ground material is weighed into a tin cup for Sulfur analyses. Carbon, nitrogen and sulfur isotope samples are measured with a continuous flow system consisting of an EA (elemental analyzer) connected to a Mass Spectrometer.

3. LIMITATIONS

When weighing out material into the tin cups, it is important not to touch the cups or sample material with bare hands, to avoid potential contamination.

4. EQUIPMENT AND SUPPLIES

4.1 Equipment

.1.1 Grinding - For small samples, use a porcelain mortar and pestle or modified coffee grinder. For larger samples, a custom-made ball mill will be used.

4.1.2 Weighing samples - Sartorius M2P microbalance (or equivalent), small spatula, small tin capsules, Plexiglas plate with 12 holes, forceps.

4.1.3 Drying oven

- 4.1.4 A continuous flow system consisting of an ANCA elemental analyzer automated sampling device connected to a HYDRA 20-20 Mass Spectrometer for (C and N) and a Isoprime continuous flow gas ratio mass spectrometer interfaced with a Carlo-Erba elemental analyzer automated sampling device.

4.2 Sampling/Storing Containers

4.2.1 Sampling containers

- 4.2.1.1 Use only new glass containers or plastic bags for invertebrates and plant material.
- 4.2.1.2 Whole fish can be placed in plastic bags. After muscle dissection, they should be placed immediately in the drying oven on aluminum or stored in clean glass or plastic containers

4.2.2 Containers for storing dried tissue

- 4.2.2.1 Dried material should be stored in well sealed 25-ml scintillation vials or equivalent.
- 4.2.2.2 Sample vials may be stored in a dessicator

5.0 PROCEDURES

5.1 Initial Sample Preparation

Fish - White muscle tissue should be dissected from the dorso-lateral (“shoulder”) region of each fish. When collecting this tissue, be sure to exclude blood, skin and scales. Be sure to generate ≥ 12 g (wet weight) of tissue.

Invertebrates - Rinse all invertebrates with DI water to remove any surficial dirt or debris. Note the total number of invertebrates in each composite. Prepare samples as described in dry weight sample prep SOP.

Algae - Remove epiphytic algae from aquatic vegetation by gently pulling it off with your hands or by using a soft toothbrush. For both phytoplankton and epiphytic algae, samples should be investigated under a microscope to determine purity and taxonomic grouping. Describe composition and note

the approximate amount of detritus, if any. Samples that have more than 5% detritus should not be used. If all samples have more than this, be sure to note it and collect pure detritus samples for comparison.

Detritus - Inspect under a microscope to ensure your samples are detritus and not algae.

Vegetation - terrestrial vegetation needs no preparation. Aquatic vegetation should be scraped with a soft toothbrush and DI water to remove any epiphytic algae.

- 5.2 Drying/Grinding, all samples - Place samples in a weighing pan or aluminum foil and mark them with the sample ID. If samples were collected in a glass jar, they can be dried in this same container. Dry samples at 55-75 °C or until a constant weight has been achieved. Crush into a fine powder with a mortar and pestle or modified coffee grinder (see dry weight sample prep SOP) or, for larger samples, the ball mill. It is important that samples not retain chunks of material, but rather be the approximate consistency of flour. Samples that are not thoroughly homogenized will create error in the analysis. Be sure to thoroughly clean the mortar/pestle after each use with DI water and a clean laboratory tissue. Use of the modified coffee grinder is described in the dry weight sample prep SOP). If using the ball mill, clean the cups, plastic lids and ball bearings in water and then place in a drying oven to remove all water before re-use. Be sure not to touch the sample powder with your hands. Use a spatula or forceps to remove ball bearings after samples have been ground. After samples are ground, place individual samples into scintillation vials (or equivalent small glass vials) and label them with the sample ID. Keep well sealed and dry until powdered samples are weighed for analysis.
- 5.3 Weighing out samples - Tare each small tin capsule prior to adding sample powder or standard material. Add the appropriate chemical standard into the first two capsules of each run. The chemical standards are a mix of ammonium sulfate and sucrose. Total N and total C are standardized against sulphanilamide. For nitrogen and carbon isotopes in samples, weigh in approximately 2 mg of dried material. For sulfur isotopes, one to three micromoles of sulfur is needed (typically, about 10 mg of material); this is weighed into a different capsule. In order to plan the correct approximate sample size for the sulfur isotopic analyses, it will be necessary to first determine the average amount of sulfur per gram of material for each consistent sample type (if not already known) and then choose sample aliquots that provide approximately 2 micromoles sulfur (typically, this is about 10 mg of material). For all capsule loadings/weighings, record the sample aliquot weight along with the sample ID number and the tray placement number in the lab notebook. The capsule will not be labeled. The only way to relate the data to the correct sample is the placement in the tray. Therefore, be extremely careful to record correct placement locations and to maintain these placements through the analysis. Using forceps, place the tin capsule into a Plexiglas holding plate with 12 holes per row. The first two samples should be the chemical standard. The

next two samples should be a biological standard. For analysis of plant material, use standardized peach leaves as the CRM. For animal material, use bovine liver standard. After you have loaded the 2 chemical standards and the 2 biological standards, load 5 capsules with successive samples. The 6th capsule should be a split of the same sample in capsule 5. After every 10th sample loaded (including sample splits but not the standards), load one additional chemical standard and one additional biological standard. After each row of 12 tin capsules is filled, use the stainless steel probe to manipulate each of the capsules into the size and shape needed for flawless drop-down into the analyzer. Carefully pick up the Plexiglas tray with the prepared samples and place over the first row on the machine sample tray. Gently push the samples through the Plexiglas into the machine tray. Repeat until all of the samples are loaded and in place. Samples are now ready for EA analysis.

- 5.4 Continuous flow analysis - There is a constant supply of reference gas passing through the Mass Spectrometer for both carbon/nitrogen and sulfur. The elemental analyzer combusts the samples in a stream of high purity oxygen and converts the carbon, nitrogen and sulfur to CO₂, NO₂ and SO₂. The oxidized gas is carried by a helium stream through a chromatography column to separate it from other gases and is then introduced into the isotope ratio mass spectrometer. The sample isotope ratio is determined relative to a pulse of high purity reference gas injected into the mass spectrometer just prior to the arrival of the oxidized gas pulse from the chromatography column. Isotope ratios are calibrated relative to the NBS reference materials. The working standard for delta 15N is N₂ gas, that for delta 13C is CO₂ gas. These two gases are calibrated against international standards, IAEA N1 ammonium sulfate (NIST 8539) and NBS22 oil (NIST 8539).

6.0 Equations

SI compositions are expressed in ‰ values which are parts per thousands difference from a standard.

$$\text{‰ X} = [(R_{\text{sample}} / R_{\text{standard}}) - 1] * 1000$$
$$R = {}^{13}\text{C}/{}^{12}\text{C}, {}^{15}\text{N}/{}^{14}\text{N}, {}^{34}\text{S}/{}^{32}\text{S}$$

7.0 Quality Assurance/Quality Control

To assure that samples are well homogenized, every 5th sample has its isotope ratios measured in duplicate as a split sample. If values are not within +/- 0.5, corrective action will be taken. The ground sample should be inspected for unhomogenized chunks of material. If the material is not finely ground, then it should be ground again into a finer powder. This sample should be run again in split/duplicate. If split values are still not within the proper range of each other, another sample should be collected and analyzed.

To assure that the continuous flow system is running properly, chemical standard with known stoichiometric values for carbon/nitrogen/sulfur and isotope ratios are used. If these values are not recovered within the correct range, then values from the samples are considered incorrect. The system must be inspected and repaired and samples must be analyzed again.

To assure that biological samples have not been contaminated and that values are within a reasonable range, biological standards are used. Since plant and animals vary, biological standards for each of these parameters need to be used. These standards have known SI ratios for carbon and nitrogen. Sulfur has no regularly used biological standard, although bovine liver (the standard for animal tissue) is used in some cases. If sample values are not within the range of the biological standards, then samples should be analyzed again. If, upon re-analysis, sample values are still out of range, then new samples should be collected and analyzed.

8.0 REFERENCES

- Fry, B. 1988. Food Web Structure on George's Bank from Stable C, N, and S Isotopic Composition. *Limnol. Oceanogr.* 33(5): 1182-1190
- Jennings, S., O. Reñones, B. Morales-Nin, N. V. C. Polunin, J. Moranta and J. Coll. 1997. Spatial Variation in the ^{15}N and ^{13}C Stable Isotope Composition of Plants, Invertebrates and Fishes on Mediterranean Reefs: Implications for the Study of Trophic Pathways. *Mar. Ecol. Prog. Ser.* 146: 109-116
- Peterson B. J. and B. Fry. 1987. Stable Isotopes in Ecosystem Studies. *Ann. Rev. Ecol. Syst.*, 18: 293-320
- Wada, E., H. Mizutani and M. Minagawa. 1991. The Use of Stable Isotopes for Food Web Analysis. *Critical Reviews in Food Science and Nutrition*, 30(3): 361-371.

UC Davis Mercury Bioaccumulation Monitoring for the California Bay-Delta Authority (CBDA)

Laboratory Organization Chart

Last revised by Darell Slotton
April 18, 2006

Darell Slotton
(P.I.)

(Project direction, planning, field collections, some sample prep., final QA)

Shaun Ayers
(Project Manager)

(Planning, field collections, sample prep., data reduction, QA)

Ron Weyand

(Laboratory Manager / Analytical Chemist)

(Sample prep., analytical chemistry, laboratory upkeep, analytical QA/QC)

(Occasional student/post-graduate help as needed)
(Sample prep., laboratory chores)

Direction flows from each project member to those listed below them:

Shaun receives direction from Darell.

Ron receives direction from Shaun or Darell.

Student helpers receive direction from Ron, Shaun, or Darell.