Quality Assurance Project Plan for the Eighteenmile Creek Area of Concern Powdered Activated Carbon Pilot Study, Baseline Benthic Community Sampling, and Baseline Fish Sampling

Contract No. EE-003700-0001

July 2012

Prepared for:

NIAGARA COUNTY SOIL AND WATER CONSERVATION DISTRICT
4487 Lake Avenue
Lockport, NY 14094

Prepared by:

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368 Pleasant View Drive
Lancaster, New York 14086

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Niagara County, New York

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The following personnel or their designees have reviewed the Quality Assurance Project Plan (QAPP) and concurred with the contents.

_____

Donna Ringel — Date
USEPA Region 2 QA Officer

_____

Alicia Reinmund-Martinez — Date
USEPA Region 2 Project Officer

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Victor DiGiacomo — Date
NCSWCD - Project Manager

_____

Kris Erickson — Date
E & E – Project Manager

_____

Marcia Galloway — Date
E & E – Quality Assurance Officer

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List of Acronyms

AOC	–	Area of Concern
AVS/SEM	–	acid volatile sulfides/simultaneously extracted metals
BUI	–	Beneficial Use Impairments
CLP	–	Contract Laboratory Procedure
COC	–	chain of custody
°C	–	degrees Celsius
CV	–	coefficient of variation
DOT	–	United States Department of Transportation
DQO	–	data quality objective
DUSR	–	data usability summary report
E & E	–	Ecology and Environment, Inc.
EDD	–	electronic data deliverable
ERDC	–	Engineering Research and Development Center
FS	–	Feasibility Study
FTP	–	file transfer protocol
GIS	–	geographic information system
GPS	–	Geographic Positioning System
GRLI	–	Great Lakes Restoration Initiative
IJC	–	International Joint Commission
LCS	–	laboratory control samples
m/s	–	meter per second
MS/MSD	–	matrix spike/matrix spike duplicate
NCSWCD	–	Niagara County Soil and Water Conservation District
NYSDEC	–	New York State Department of Environmental Conservation
NYSDOH	–	New York State Department of Health
PAC	–	powdered activated carbon
PARCC	–	precision, accuracy, representativeness, completeness, and comparability
PCB	–	polychlorinated biphenyl
PDF	–	portable document format
QA/QC	–	quality assurance/quality control
QAPP	–	Quality Assurance Project Plan
REIC	–	REI Consultants, Inc.
RI	–	Remedial Investigation
RPD	–	relative percent difference
SDG	–	Sample Delivery Group
SOP	–	Standard Operating Procedure

Additional Lists (cont.)

USACE	–	United States Army Corps of Engineers
USEPA	–	United States Environmental Protection Agency

Additional Approvals List

Party	Affiliation and Title	Approval Signature	Revision	Date
QAPP for Powdered Activated Carbon Pilot Study, Baseline Benthic Community Sampling, and Baseline Fish Sampling				
Justin Zoladz	E & E Field Team Manager		1	
Lisa Domenighini	ALS-Kelso Project Manager		1	
John Williams	Aquatec Project Manager		1	
Ed Kirk	REI Consultants Director		1	

Key:
E & E = Ecology and Environment, Inc.
QAPP = Quality Assurance Project Plan
QA = Quality Assurance

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Key:

CAS	=	Columbia Analytical Services
E & E	=	Ecology Environment Engineering P.C.
NCSWCD	=	Niagara County Soil and Water Conservation District
QAPP	=	Quality Assurance Project Plan
QA	=	Quality Assurance
USEPA	=	United States Environmental Protection Agency

1 Project Management

This Quality Assurance Project Plan (QAPP) has been prepared by Ecology and Environment, Inc. (E & E) for the Niagara County Soil and Water Conservation District (NCSWCD) under Contract No. EE-003700-0001 to support a Powdered Activated Carbon (PAC) Pilot Study, Baseline Benthic Community Sampling, and Baseline Fish Sampling at Eighteenmile Creek, Niagara County, New York. This QAPP presents the policies, organization, objectives, functional activities, and specific quality assurance/quality control (QA/QC) procedures that will be employed by E & E to ensure that all technical data generated for the work are accurate, representative, and ultimately capable of withstanding rigorous scientific scrutiny. The project is funded by a Great Lakes Restoration Initiative (GRLI) grant to the NCSWCD. Ultimately, the data collected under this QAPP will be used to support development of a Feasibility Study (FS) for the Eighteenmile Creek Area of Concern (AOC) and upstream source areas and help to better understand Beneficial Use Impairments (BUIs) at the site.

This QAPP was developed in accordance with the four major sections provided in the USEPA QAPP guidance document (USEPA 2006a): *Project Management, Data Generation and Acquisition, Assessment and Oversight, and Data Validation and Usability*. Any required elements of the QAPP guidance that are not applicable to the project work described herein are indicated in the individual sections. Appendices to the QAPP provide additional detail on project procedures and supporting material. Field procedures are included in Appendix A. Laboratory procedures are included in Appendix B. Supporting documents including checklists and forms are included in Appendix C.

1.1 Project Organization

The organizational chart for the project is presented on Figure 1-1. The project is led by the NCSWCD. The NCSWCD Project Manager and QA Officer are responsible for review and approval of the QAPP. The NCSWCD Project Manager will direct the work activities of the E & E project team, address potential deviations from planned activities, and approve corrective actions.

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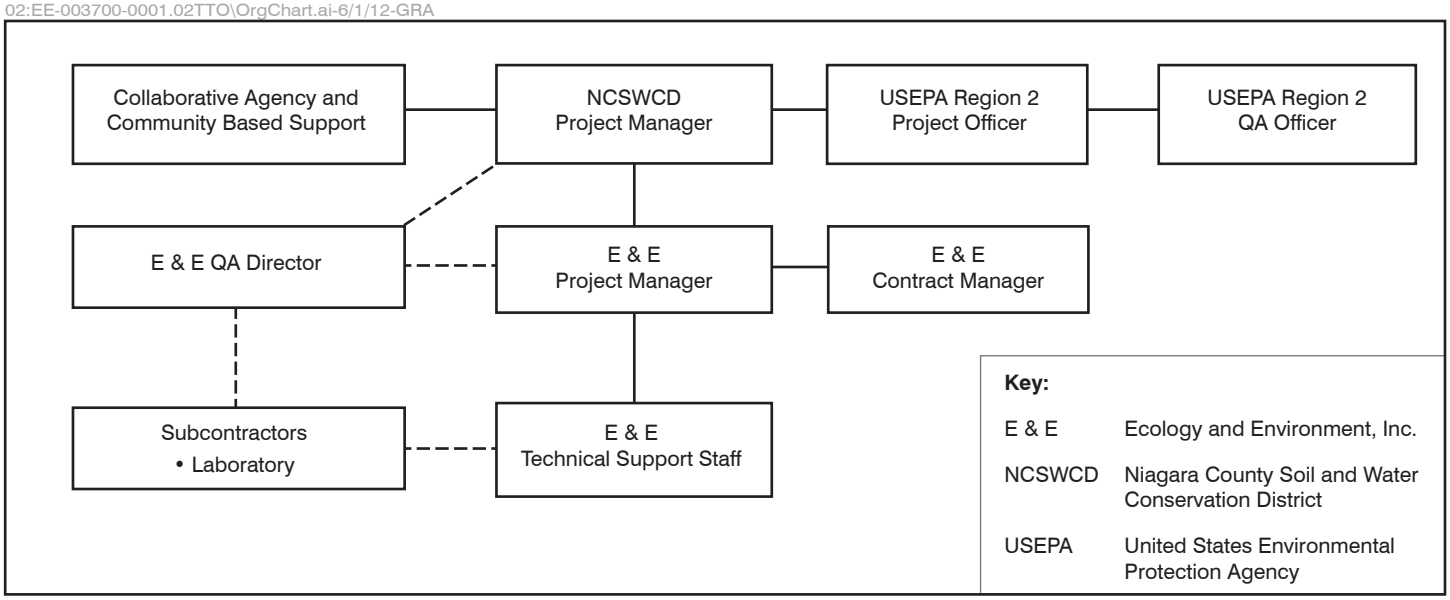

Figure 1-1 Eighteenmile Creek Powdered Activated Carbon Pilot Study and Baseline Benthic and Fish Sampling Project Organization Chart

The roles and specific QA responsibilities of key project personnel are described in Table 1-1.

Table 1-1 Key Project Personnel

Key Personnel	Affiliation and Role	Project Responsibilities
Victor DiGiacomo	NCSWCD Project Manager	■ Direct the work activities of the E & E project team ■ Approve QAPP ■ Address potential deviations from planned activities ■ Approve corrective actions
Kris Erickson	E & E Project Manager	■ Coordinate contract activities with the NCSWCD Project Manager ■ Oversee day-to-day activities, including technical and administrative operations ■ Review and approve final reports and other work products. ■ Approve, implement and distribute the QAPP. ■ Train project team on QAPP.
Marcia Galloway	E & E Quality Assurance Officer	■ Remain independent of day-to-day, direct project involvement ■ Ensure that project QA/QC requirements are met ■ Independent technical review of project deliverables ■ Approve QAPP ■ Resolve any QA/QC problems, disputes, or deficiencies and approve corrective actions
Sean Meegan, Carl Mach, Robert Singer	E & E Task Leads E & E	■ Liaisons between the Project Manager and the project team regarding QC implementation, updates, and corrective actions for specific tasks ■ Prepare QAPP sections on Data Generation and Acquisition ■ Assist PM in directing field staff ■ Assist QA Officer in coordinating with and directing subcontractors ■ Data interpretation and report preparation
Justin Zoladz, John Hallock, Joe Galati	Field Staff	■ Perform field tasks in accordance with QAPP and SOPs
Lisa Casey	E & E GIS Analyst	■ Maintain spatial layers and overall geo-database integrity ■ Provide the GIS-related outputs for reports and the Web site

Table 1-1 Key Project Personnel

Key Personnel	Affiliation and Role	Project Responsibilities
Bryan Kroon	E & E Project Chemist	■ Coordinate with laboratories ■ Package and ship samples ■ Review data from laboratories ■ Import field and laboratory data to project database in Equis ■ Provide reports and client electronic data deliverables (EDDs)
Jan Brick	E & E Publications Staff	■ Maintain document control and security and long-term storage of related document project files
Alicia Reinmund-Martinez	USEPA Project Officer	■ Review project QAPP and technical reports ■ Provide constructive technical feedback to project team
Donna Ringel	USEPA Quality Assurance Officer	■ Review project QAPP and technical reports ■ Provide constructive technical feedback to project team
Subcontractors		
L. Domenighini (ALS-Kelso), J. Williams (Aquatec), E. Kirk (REIC)	Laboratory Project Manager	■ Coordinate sample receipt, analysis and report generation ■ Ensure methods are performed in accordance with QAPP

Key:
Aquatec = Aquatec Biological Sciences
CAS = Columbia Analytical Services
REIC = REI Consultants

1.2 Problem Definition/Background

1.2.1 Problem Definition

In 1987, the International Joint Commission (IJC) identified 43 AOCs in the Great Lakes Basin where the beneficial uses of the water body were considered impaired. Eighteenmile Creek was identified as one of the 29 United States AOCs. The creek has been polluted by past industrial and municipal discharges, the disposal of waste, and the use of pesticides. Currently, there are five documented BUIs at the Eighteenmile Creek AOC: (1) restrictions on fish and wildlife consumption; (2) degradation of fish and wildlife populations; (3) bird or animal deformities or reproductive problems; (4) degradation of benthos; and (5) restrictions on dredging activities (USEPA 2010a). These five BUIs are largely driven by elevated levels of polychlorinated biphenols (PCBs) in sediment and fish (E & E 2011). Table 1-2 lists the site-specific BUI delisting criteria developed by the NCSWCD for the Eighteenmile Creek system.

Table 1-2 Beneficial Use Impairments and Delisting Criteria for the Eighteenmile Creek AOC

BUI	BUI Status	Delisting Criteria
1. Restrictions on Fish and Wildlife Consumption	Impaired	There are no AOC-specific fish and wildlife consumption advisories issued by New York State; **AND**
		Contaminant levels in fish and wildlife must not be due to contaminant input from the watershed upstream of Burt Dam
3. Degradation of Fish and Wildlife Populations	Impaired	Fish and wildlife diversity, abundance and condition are statistically similar to diversity, abundance and condition of populations at non-AOC control sites; **AND**
		PCB levels in bottom-dwelling fish do not exceed the critical PCB tissue concentration for effects on fish (440 micrograms per kilogram of weight; Dyer et al. 2000)
5. Bird or Animal Deformities or Reproduction Problems	Impaired	No reports of wildlife population deformities or reproductive problems from wildlife officials above expected natural background levels; **AND**
		Contaminant levels in bottom-dwelling fish do not exceed the level established for the protection of fish-eating wildlife (NYSDEC Fish Flesh Criteria); **OR**
		In the absence of fish data, the toxicity of sediment-associated contaminants does not exceed levels associated with adverse effects on wildlife (NYSDEC Fish & Wildlife Bioaccumulation Sediment Criteria)
6. Degradation of Benthos	Impaired	Benthic macroinvertebrate communities are “non-impacted” or “slightly impacted” according to NYSDEC indices; **OR**
		In the absence of NYSDEC data, riffle habitats require benthic macroinvertebrate communities with a species richness higher than 20, EPT richness greater than 6, a biotic index value greater than 4.51, and a percent model affinity greater than 50; **OR**
		In the absence of benthic community data, this use will be considered restored when the level of toxic contaminants in sediments is not significantly higher than controls.
7. Restrictions on Dredging Activities	Impaired	When contaminants in AOC sediments (located within the actual or potential dredging areas identified for the improvement of ship navigation) do not exceed standards, criteria, or guidelines such that there are restrictions on dredging or disposal activities.

Source: USEPA 2010a

Key:
AOC = Area of Concern
BUI = Beneficial Use Impairment
EPT = Ephemeroptera, Plecoptera, and Trichoptera
NYSDEC = New York State Department of Environmental Conservation
PCB = polychlorinated biphenyl

Both human and ecological receptors using the Eighteenmile Creek system may be at risk from PCBs and perhaps other chemicals in fish based on recent investigations (E & E 2009a) and current fish consumption advisories (NYSDOH 2011). Elevated levels of PCBs in fish in Eighteenmile Creek appear to be the result of bioaccumulation from sediment (USACE 2004a, b; von Stackelberg and Gustavson 2012). Recent sediment data from the Remedial Investigation (RI) for Eighteenmile Creek show that surface sediment PCB levels are greater in the portion of the creek near the source areas in Lockport, New York, compared with downstream reaches (E & E 2012). Source areas along the creek in Lockport were characterized by the New York State Department of Environmental Conservation (NYSDEC 2006) and E & E (2009a). Remediation of upstream sources areas and contaminated sediment throughout the creek is necessary to eliminate BUIs in the Eighteenmile Creek system and eventually delist this Great Lakes AOC (E & E 2011).

1.2.2 Background

The Eighteenmile Creek AOC is located in Niagara County, New York (see Figure 1-2). The creek flows generally north through central Niagara County and discharges via Olcott Harbor into Lake Ontario, approximately 18 miles east of the mouth of the Niagara River. The AOC includes Olcott Harbor and extends upstream to the farthest point at which backwater conditions exist during Lake Ontario's highest monthly average lake level (see Figure 1-2). This point is located just downstream of Burt Dam, approximately 2 miles south of Olcott Harbor. This portion of the watershed is a unique gorge habitat that attracts recreational boaters, anglers, birders, and waterfowl hunters.

Only a small portion of the Eighteenmile Creek basin was originally designated an AOC by the IJC. However, for two reasons, since the Eighteenmile Creek RAP process began, the AOC has been considered the impact area and the upper watershed as the source area (NYSDEC 1997). First, except for potential impacts from agricultural operations adjacent to the current AOC boundary, there are no documented sources or source areas of contamination within the AOC. Second, various investigations conducted over the past 35 years have suggested that contaminants may enter the AOC from upstream areas. Specifically, PCBs, copper, lead, and other metals have been found in creek sediment and bank fill in Lockport, New York, at concentration well above applicable NYSDEC standards, indicating that contaminant sources exist in this area (NYSDEC 2006, E & E 2009a and 2012). Other contaminant source areas may exist along the creek between Lockport and the AOC (NYSDEC 2001).

Additional information regarding the characteristics of the Eighteenmile Creek AOC and watershed are available in the *Eighteenmile Creek State of the Basin Report* (E & E 2007), *Beneficial Use Impairment (BUI) Investigation Report for Eighteenmile Creek* (E & E 2009b), *Sediment Remedial Investigation Report* (E & E 2012), and additional publications and factsheets available from the Eighteenmile Creek Remedial Action Plan (RAP) website (www.eighteenmilecreekrap.com). Specifically, the BUI Investigation listed above evaluated three of the BUIs which involved data collection on: (1) fish diversity, abundance, and condition; (2) wildlife (birds, mammals, amphibians) diversity and abundance; (3) concentrations of PCB and dioxins/furans in brown bullheads; and (4) prevalence of external, internal, and liver tumors in brown bullheads.

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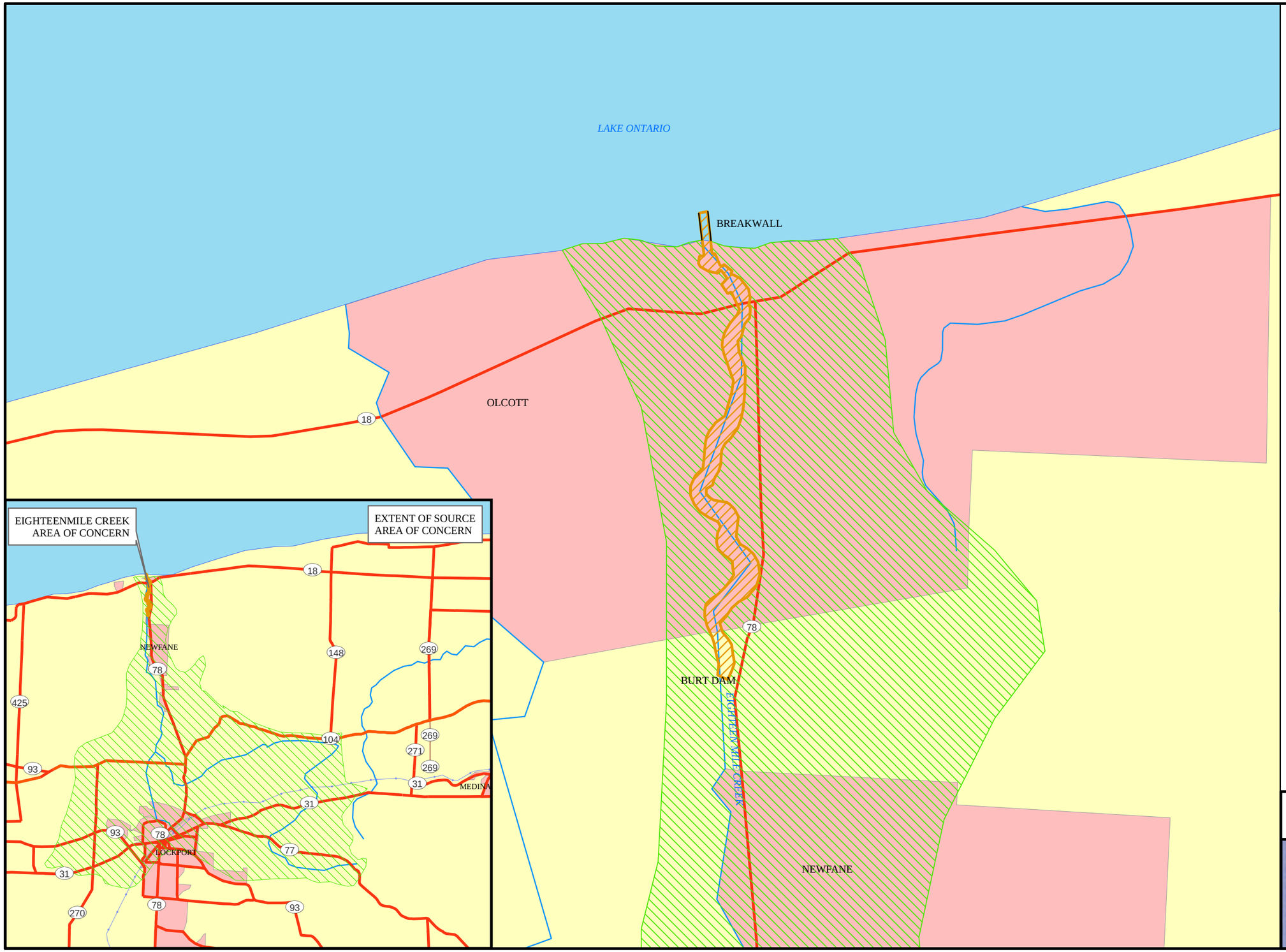

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LEGEND

- MAJOR ROADS
- SURFACE WATER
- URBAN AREAS
- EXTENT OF AREA OF CONCERN (EIGHTEENMILE CREEK REMEDIAL ACTION PLAN, AUGUST 1997)
- EXTENT OF SOURCE AREA OF CONCERN (EIGHTEENMILE CREEK REMEDIAL ACTION PLAN, AUGUST 1997)

NOTES:

AREA OF CONCERN BOUNDARY AS APPROVED BY THE NEW YORK STATE DEPARTMENT OF ENVIRONMENTAL CONSERVATION ON JULY 27, 2005.

THE SOURCE AREA OF CONCERN DESIGNATED FOR THE EIGHTEENMILE CREEK AREA OF CONCERN IS DEFINED IN THE 1997 REMEDIAL ACTION PLAN AS THE EIGHTEENMILE CREEK WATERSHED, AND WAS DERIVED FROM FIGURE 2.2 OF THE 1997 REMEDIAL ACTION PLAN.

SOURCE: MODIFIED FROM THE NEW YORK DEPARTMENT OF ENVIRONMENTAL CONSERVATION 1997, AND ESRI, 2005.

0 1,000 2,000 Feet

EIGHTEENMILE CREEK, NEW YORK
AREA OF CONCERN

Great Lakes National Program Office

EPA

Figure 1-2 Eighteenmile Creek Area of Concern

In 2010 and 2011, a strategic plan for delisting BUIs at the Eighteenmile Creek AOC was developed (E & E 2011). The strategic plan identified actions that could be undertaken to address the principal cause of BUIs in the Eighteenmile Creek system (i.e., PCBs in sediment and fish). The actions identified in the strategic plan included active remediation of source areas; remediation of in-stream contaminated sediments; development of a feasibility study (FS) for the site; investigation of novel approaches for reducing bioavailability of PCBs in situ; baseline sampling of fish and the benthic community prior to remedial work; and other measures.

1.3 Project Description

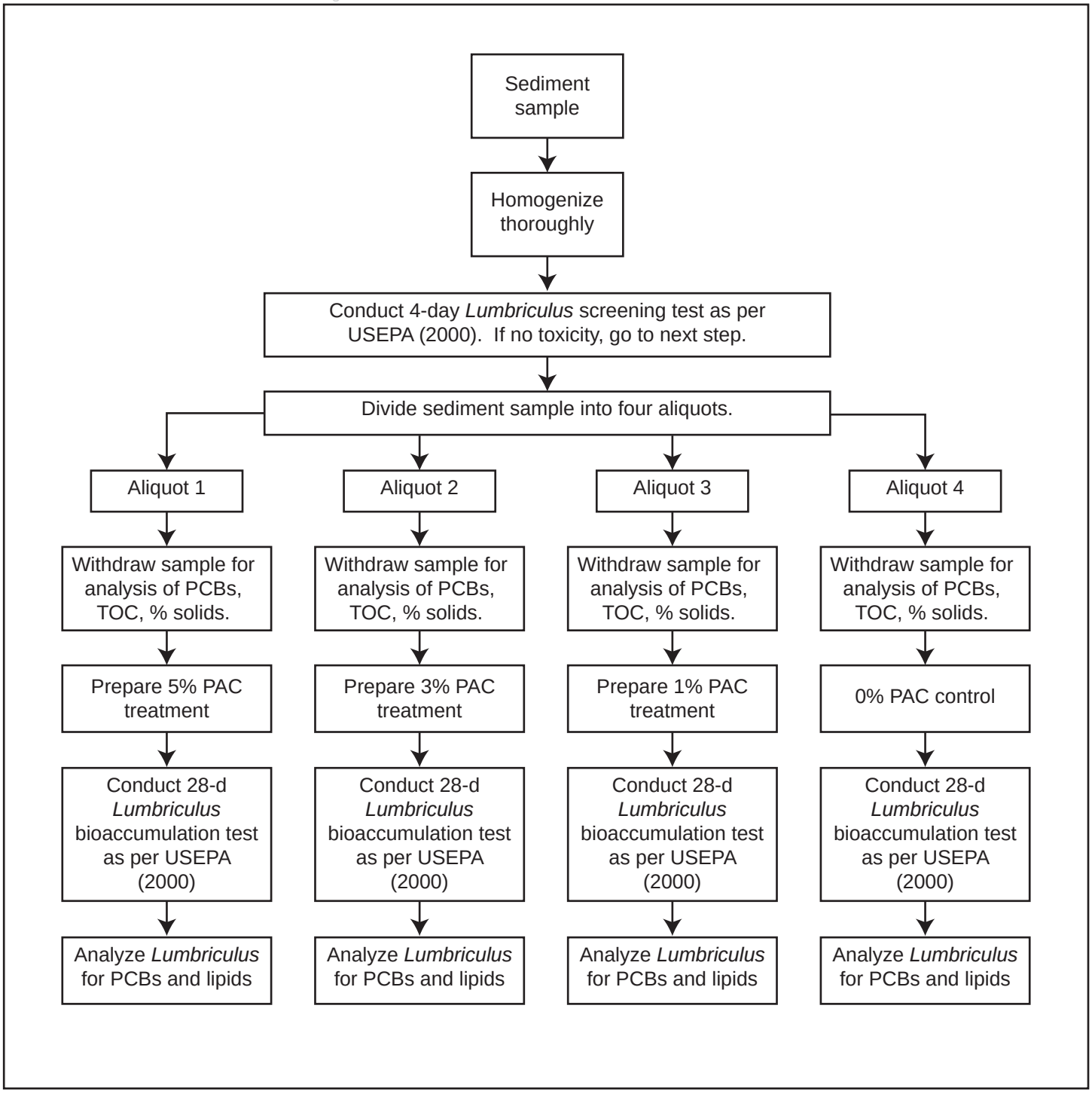
This QAPP describes three investigations that are designed to address three actions recommended in the strategic plan for BUI delisting for Eighteenmile Creek. These projects are: (1) pilot study on use of powdered activated carbon (PAC) to reduce PCB bioavailability in sediment; (2) baseline fish sampling; and (3) baseline benthic-community sampling. These projects are described in turn below.

1.3.1 PAC Pilot Study

The objective of this investigation is to evaluate the effectiveness of using PAC to reduce bioavailability of PCBs in Eighteenmile Creek sediment. Up to 90% reduction in PCB bioavailability has been observed at sites where this method has been used (Alcoa 2010). The five BUIs at Eighteenmile Creek are all ultimately linked to high PCBs levels in sediment (see Section 1.2). If the pilot study proves to be successful (i.e., if PAC is effective in reducing PCB bioavailability in Eighteenmile Creek sediment), then application of this technique *in situ* has the potential to contribute to delisting of these BUIs. The results of this project will be incorporated into the Eighteenmile Creek FS (expected to be initiated during the summer of 2012). Sediment treatment with PAC may be useful in Eighteenmile Creek as a polishing step in areas where sediment dredging is implemented and/or as the principal means of sediment remediation in areas that are difficult or infeasible to access for dredging.

This investigation will include a laboratory bench-top pilot study with sediment from Eighteenmile Creek. Sediments representing a range of PCB concentrations will be collected and augmented with a range of PAC concentrations (see Table 1-3 for experimental design and Figure 1-3 for flowchart). Bioaccumulation in each PAC treatment will be determined by USEPA (2000) Test Method 100.3 (28-day *Lumbriculus variegates* Bioaccumulation Test for Sediments). For comparison, bioaccumulation in untreated (control) creek sediment from Eighteenmile Creek also will be evaluated. Sediment samples will be analyzed for PCB Aroclors and congeners (8082 list) and total organic carbon and the test organisms will be analyzed for PCB Aroclors and congeners (8082 list) and percent lipids. Analytical methods and quality objectives and criteria are presented in Section 2.4. Bioaccumulation will be evaluated by comparing PCB levels in test organisms among treatments and by calculating and comparing biota-sediment accumulation factors among treatments.

02:EE003700-0001-02\Flowchart Bioaccumulation Eighteenmile Creek.ai-6/4/12-GRA



SOURCE: Ecology and Environment, Inc. 2012

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Figure 1-3 Flowchart for Bioaccumulation Testing with Eighteenmile Creek Sediment Amended with Powdered Activated Carbon (PAC)

Table 1-3 Experimental Design for Eighteenmile Creek Powdered Activated Carbon (PAC) Pilot Study

Sediment Sample	Location	Treatment	Possible PAC Treatment Concentration
1	Downstream from Burt Dam	High PAC	5%
		Medium PAC	3%
		Low PAC	1%
		No PAC	0%
2	Between Burt and Newfane Dams	High PAC	5%
		Medium PAC	3%
		Low PAC	1%
		No PAC	0%
3	Upstream from Newfane Dam	High PAC	5%
		Medium PAC	3%
		Low PAC	1%
		No PAC	0%

Source: E & E 2012

Key:
PAC = Powdered activated carbon

E & E examined the relationship between sample size, coefficient of variation (CV), and statistical power and confidence, with respect to the PAC study design. It should be noted that the cost for bioaccumulation testing and chemical analysis for each sediment sample included in the PAC pilot study is high, therefore adding additional samples to the study was prohibited by cost. With three samples (one per reach), the PAC pilot study will be able to detect a 50% difference in the *Lumbriculus* PCB concentration between the control and treatments with a statistical power of 90% and confidence of 80% if the CV in the *Lumbriculus* PCB concentration is 25% (see Table 1-4). E & E considers these to be reasonable statistical performance goals for the present study. If the CV is less than 25%, a smaller difference between the control and treatments can be detected (see Table 1-4).

Specific sediment sampling locations for the PAC pilot study are listed in Table 1-5 and shown in Figures 1-4 to 1-6. One sampling location was selected in each of the three main reaches of the creek: (1) below Burt Dam; (2) between Newfane and Burt Dams; and (3) upstream from Newfane Dam. In addition, the sampling locations were selected to encompass a range of total PCBs concentration of approximately an order of magnitude (see Table 1-5). Existing sediment PCB data for the creek (tabulated in E & E 2012) were reviewed in the selection process. Because a large sediment volume (approximately 10 gallons) will be required from each location, a composite sample will be collected by taking multiple hauls with an Ekman or Ponar dredge within each sampling area. Sampling methods are described further in Section 2.2 (Sampling Methods).

Table 1-4 Relationship Between Measures of Statistical Performance and Number of Samples Required for the PAC Pilot Study

Coefficient of Variation (%)	Power (%)[b]	Confidence Level (%)[b]	Number of Samples Required to Identify Differences of 10% to 100% Between Treatment and Control[a]					
			10%	15%	20%	30%	50%	100%
10%	90	80	9	4	3	1	1	0
15%	90	80	21	9	5	3	1	1
20%	90	80	36	16	9	4	2	1
25%	90	80	57	25	14	7	3	1
30%	90	80	82	36	21	9	4	1
40%	90	80	145	65	36	16	6	2
50%	90	80	226	101	57	25	9	3
60%	90	80	325	145	82	36	13	4
70%	90	80	442	197	111	49	18	5
80%	90	80	578	257	145	65	23	6
90%	90	80	731	325	183	82	30	8

Notes:
[a] One tailed, two-sample t-test, treatment versus control. Based on USEPA (1992, Appendix IV).
[b] 80% confidence and 90% power are minimum performance values from USEPA (1992, Exhibit 68).

Table 1-5 PAC Study Sediment Sampling Locations

Reach	Sample Area Description	Feet Mark[a]		PCB Concentration (mg/kg)[b]		
		Downstream	Upstream	n	Range	Average
1	Between Burt Dam and Lake Ontario	75500	73677	6	0.17 - 1.96	0.9
3	Upstream end of Burt Dam pool	60756.9	60734.2	8	0.11 - 8.3	2.9
5	Newfane Dam Pool	52060.8	51122.4	11[c]	0.01 - 0.25	0.1

Notes:
[a] Feet downstream from Erie Canal, Lockport, New York.
[b] Remedial Investigation Report (E & E 2012, March).
[c] Excluding one outlier from 1994 which does not reflect current conditions.

Key:
mg/kg = milligrams per kilogram
PCB = polychlorinated biphenyl

1.3.2 Baseline Sampling of Fish from Different Trophic Levels

The objectives of this investigation are to: (1) collect and analyze fish and crayfish for PCB Arolcors and congeners to support development of a baseline dataset regarding PCB levels in fish from different trophic levels throughout Eighteenmile Creek, (2) provide data that may be used to re-evaluate the status of fish consumption advisories for Eighteenmile Creek, and (3) generate data that may be used to further populate the Trophic – Trace Model begun by the United States Army Corps of Engineers (USACE) – Engineering Research and Development Center (ERDC) (von Stackelberg and Gustavson 2012).

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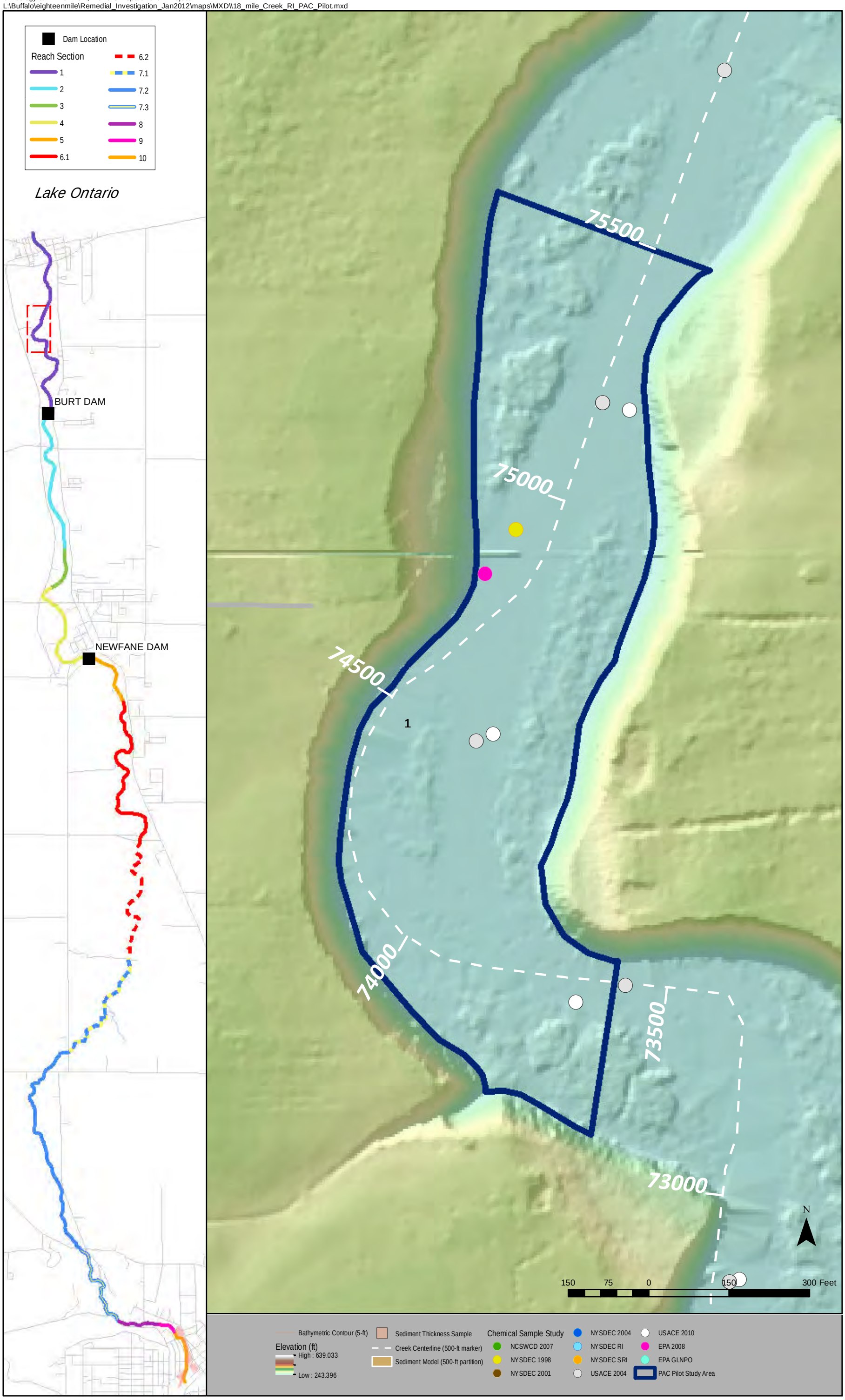

Figure 1-4. PAC Pilot Study Sample Area 1

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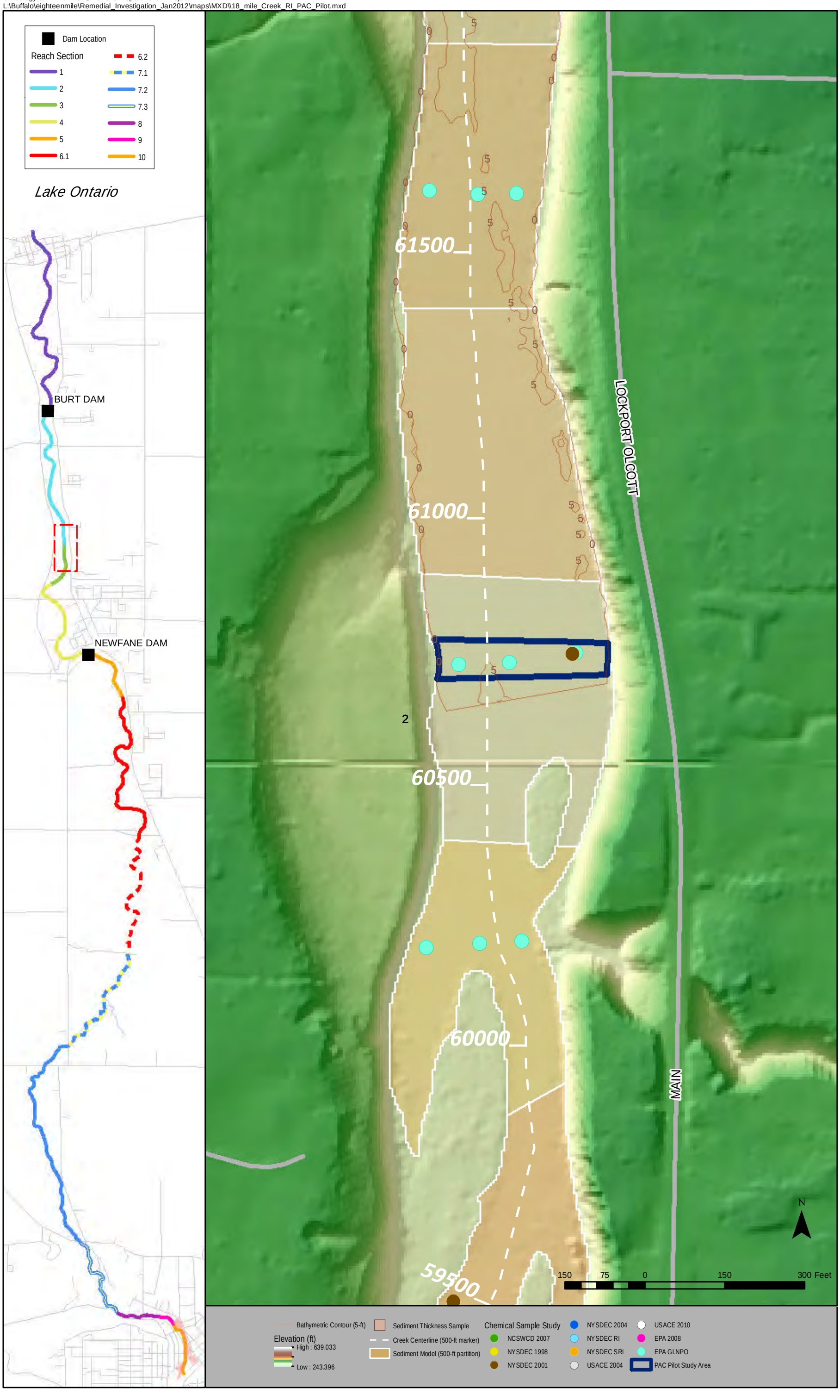

Figure 1-5. PAC Pilot Study Sample Area 2

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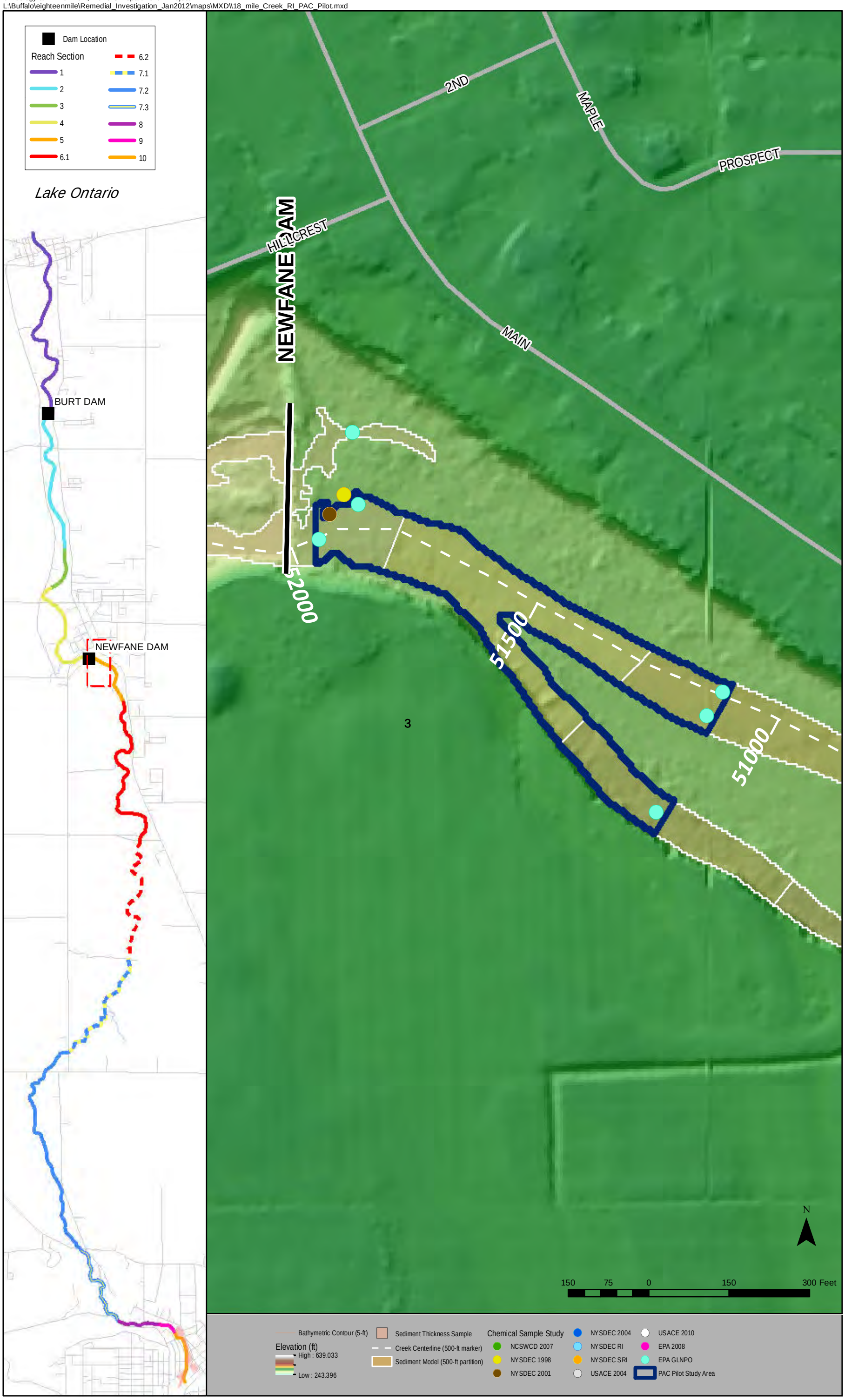

Figure 1-6. PAC Pilot Study Sample Area 3

The investigation will entail collecting forage fish composite samples, game fish fillet and whole-body samples, and crayfish composite samples from three reaches of Eighteenmile Creek: (1) below Burt Dam; (2) between Newfane and Burt Dams; and (3) upstream from Newfane Dam. Target and alternate species, total numbers of samples, and other sampling details are provided in Table 1-6. The numbers of samples per reach are provided in Table 1-7. The fish and crayfish samples will be analyzed for PCB Aroclors and congeners (EPA 8082 list), lipids, and percent moisture. Analytical methods and quality objectives and criteria are presented in Section 2.4. The work will be completed according to the New York State Department of Health (NYSDOH), NYSDEC, and United States Environmental Protection Agency (USEPA) standards to ensure data usability by all interested parties.

Table 1-6 Target and Alternate Species, Numbers of Samples, and Other Sampling Details for Eighteenmile Creek Baseline Fish Sampling Project

Target Species	Alternate Species	Life Stage	Sample Type	Number of Samples	Number of Individuals per Sample	Total Number of Individuals[a]
Brown bullhead (*Ameiurus nebulosus*)	Sucker (*Catostomus* spp.) or carp (*Cyprinus carpio*)	Adult	Fillet	15	1	15
Pumpkinseed (*Lepomis gibbosus*)	Other sunfish (*Lepomis* spp.), golden shiner (*Notemigonus crysoleucas*) or fathead minnow (*Pimephales promelas*)	Juvenile or adult	Whole-body composite	15	10	150
Largemouth bass (*Mircopterus salmoides*)	Smallmouth bass (*Mircopterus dolomieu*)	Adult	Whole-body	5	1	5
Crayfish (*Orconectes* spp.)	*Cambarus* spp.	Adult or juvenile	Whole-body composite	9	10	90

Note:
[a] For fish and crayfish, sex will be recorded if it can be readily determined without dissection, which could compromise sample integrity.

To provide data relevant to the delisting criteria for BUIs No. 3 and No. 5, the brown bullhead (*Ameiurus nebulosus*) will be the target game fish species in all three reaches of the creek (see Table 1-7); delisting criteria for both BUIs require that contaminant levels in bottom-dwelling fish are less than applicable standards (see Table 1-2). Also, because the brown bullhead and other bottom-dwelling fish are consumed by people, contaminant data for bottom-dwelling species also can be used to evaluate the delisting criteria for BUI No. 1 (see Table 1-2). An earlier study by E & E (2009b) sampled brown bullhead below Burt Dam and more recent work by von Stackelberg and Gustavson (2012) sampled this species below and above Burt Dam. Hence, it is expected that brown bullhead will be available throughout the creek. However, if

they are not, another bottom-dwelling species, such as white sucker (*Catostomus commersonii*), will be collected. This species was observed above the Newfane Dam during sediment sampling conducted in 2010 and 2011 for the Eighteenmile Creek RI (E & E 2012).

Table 1-7 Number of Samples by Reach for Eighteenmile Creek Baseline Fish Sampling Project

Reach	Description	Target Species[a]	Sample Type	Number
1	Lockport to Newfane Dam	Brown Bullhead	Fillet	5
		Pumpkinseed	Whole-body composite	5
		Largemouth bass	Whole-body	5
		Crayfish	Whole-body composite	3
2	Newfane to Burt Dam	Brown Bullhead	Fillet	5
		Pumpkinseed	Whole-body composite	5
		Largemouth bass	Whole-body	0
		Crayfish	Whole-body composite	3
3	Burt Dam to Lake Ontario	Brown Bullhead	Fillet	5
		Pumpkinseed	Whole-body composite	5
		Largemouth bass	Whole-body	0
		Crayfish	Whole-body composite	3
			TOTAL	**44**

Note:
[a] See Table 1-6 and Section 1.3.2 for Latin names, life stage, alternate species, rationale, and other sampling details.

Regarding forage fish, E & E will collect juvenile (approximately 4 inches total length) pumpkinseed (*Lepomis gibbosus*). This species was collected recently for the Trophic-Trace model for Eighteenmile Creek (von Stackelberg and Gustavson 2012) and is expected to be available throughout the creek. Pumpkinseeds are a common forage species for predatory fish, such as largemouth bass (*Micropterus salmoides*) and northern pike (*Esox lucius*), both of which have been observed in the creek. Additionally, when sunfish reach larger sizes, they may be targeted by anglers for consumption. If pumpkinseeds are not sufficiently plentiful, other sunfish (*Lepomis* spp.), golden shiners (*Notemigonus crysoleucas*), fathead minnows (*Pimephales promelas*), or creek chubs (*Semotilus atromaculatus*) will be collected. Every effort will be made to collect single-species composite forage fish samples.

Lastly, to provide data that may be used to refine the Trophic-Trace model for Eighteenmile Creek (von Stackelberg and Gustavson 2012), three crayfish composite samples will be collected from each of the three reaches of Eighteenmile Creek, and five largemouth bass whole-body samples will be collected from the creek above Newfane Dam (see Table 1-7). Crayfish were found to be an important component of the diet of largemouth bass in sampling conducted for the Trophic-Trace model for Eighteenmile Creek, but have not been sampled previously in any reach of the creek. To our knowledge, largemouth bass have not been sampled previously from the creek above Newfane Dam. Hence, collecting crayfish and largemouth bass samples as part of the current investigation should help fill data gaps related to modeling food-chain transfer of chemicals in Eighteenmile Creek.

The following provides the rationale supporting the justification for the number of bullhead and pumpkinseed samples per reach:

- Five brown bullhead samples per reach are adequate to detect a 10% difference between the bullhead PCB concentration and PCB fish-tissue criterion with a statistical power of 90% and confidence of 80% given the variability of bullhead PCB data observed in previous studies; the CV for log-transformed total PCB data for brown bullheads collected from the creek in 2007 was approximately 10% (E & E 2009) (see Table 1-8). The statistical performance values used in the calculation (90% power, 80% confidence, and minimum detectable relative difference of 10 to 20%) are the minimum values recommended by the USEPA (1992).

- The CV for total PCBs in pumpkinseed composite samples collected from the creek in 2010 (von Stackelberg and Gustavson 2012) was similar to the CV for total PCBs in the 2007 brown bullhead samples (approximately 10%). Hence, the sample size proposed in the draft QAPP for pumpkinseed (five composite samples per reach) also should be adequate to detect a 10% difference between the fish PCB concentration and PCB fish-tissue criterion with a statistical power of 90% and confidence of 80% (see Table 1-8).

The bullhead and pumpkinseed samples represent the core of the baseline fish sampling program for Eighteenmile Creek. It is anticipated that these two species will be collected again after remedial actions are undertaken to evaluate changes in fish PCB levels over time.

As noted above, the crayfish and largemouth bass samples for PCB analysis will be collected to support the Eighteenmile Creek Trophic Trace model (von Stackelberg and Gustavson 2012). Additional justification for the number of largemouth bass and crayfish samples per reach is provided below:

- As noted above, largemouth bass are being collected only upstream from Newfane Dam because this reach has not been sampled previously for largemouth bass. Largemouth bass were collected from the lower two reaches of the creek in 2010 (von Stackelberg and Gustavson 2012). The total PCB concentration in largemouth bass collected in 2010 (von Stackelberg and Gustavson 2012) was two times more variable than the total PCB data for brown bullhead and pumpkinseed from the creek. Hence, for largemouth bass, the smallest difference between the fish PCB concentration and fish-tissue criterion that can be detected is 20 to 30% with a sample size of five fish (see Table 1-8). E & E considers this to be a reasonable minimum detectable difference for risk assessment purposes.

- Crayfish will be collected and analyzed for PCBs to fill a data gap identified during development of the Trophic Trace model for Eighteenmile Creek (von Stackelberg and Gustavson 2012). Crayfish were found to be a significant prey item for largemouth bass in Eighteenmile Creek. However, there are no PCB data for crayfish from any reach of the creek. If the CV for total PCBs in crayfish is similar to the CV for total PCBs in bullhead (10%), then three composite crayfish samples per reach will be adequate to detect a

difference of 30% between the crayfish PCB concentration and tissue criterion for PCBs with a statistical power of 90% and confidence of 80% (see Table 1-8).

Table 1-8 Relationship Between Measures of Statistical Performance and Number of Samples Required for Baseline Fish Sampling

Coefficient of Variation (%)	Power (%)[b]	Confidence Level (%)[b]	Number of Samples Required to Identify Differences of 10% to 100% Between Site Fish-Tissue Concentration and Fish-Tissue Criterion[a]					
			10%	15%	20%	30%	50%	100%
10%	90	80	5	2	1	1	1	0
20%	90	80	18	8	5	2	1	1
30%	90	80	41	18	11	5	2	1
40%	90	80	73	32	18	8	3	1
50%	90	80	113	50	29	13	5	1
60%	90	80	163	73	41	18	7	2
70%	90	80	221	99	56	25	9	3
80%	90	80	289	129	73	32	12	3
90%	90	80	366	163	92	41	15	4

Notes:
[a] One tailed, one-sample t-test, site versus criterion. Based on USEPA (1992, Appendix IV).
[b] 80% confidence and 90% power are minimum performance values from USEPA (1992, Exhibit 68).

As noted in Table 1-6, 10 individual juvenile pumpkinseed sunfish will be included in each pumpkinseed composite sample. Similarly, 10 individual crayfish will be included in each crayfish composite sample. The number of individuals per composite samples was selected to provide adequate sample mass for analysis. When a full suite of hydrophobic organic contaminants (e.g., pesticides and PCBs) are measured, composite sample mass should be at least 200 grams (USEPA 2000). Because only PCBs are measured for the current investigation, a lower sample mass is acceptable. Nonetheless, the compositing strategy in this QAPP is designed to provide 200-gram composite samples. For example, juvenile pumpkinseeds (4-inch length) weigh approximately 20 grams each. Hence, a composite sample of 10 fish will weigh approximately 200 grams. Similarly, adult crayfish also weigh approximately 20 grams. If it is not possible to include 10 pumpkinseeds or 10 crayfish per composite sample, or if the average size of individual pumpkinseeds or crayfish is smaller than expected, a smaller sample mass (down to 100 grams) still will provide adequate sample mass for analysis for the present investigation.

1.3.3 Baseline Benthic Community Sampling

The objective of this investigation is to evaluate the current condition of the benthic community in the Eighteenmile Creek AOC. The results will be used for two purposes: (1) as a baseline against which future changes in the benthic community can be measured and (2) to reevaluate the status of the BUI No. 6 (Degradation of Benthos) within the Eighteenmile Creek AOC. The sediment quality triad approach will be employed; this approach relies on concurrently evaluating sediment chemistry, sediment toxicity, and benthic community composition to draw a

conclusion regarding the overall health of the benthic community. Unlike the previous two studies, all samples for evaluation of the benthic community will be collected between Burt Dam and Lake Ontario because the delisting criteria for BUI No. 6 are focused on the AOC proper, not on upstream source areas (see Table 1-2).

E & E will use NYSDEC methods for biological monitoring of surface waters in New York State for the benthic collection effort (Bode et al. 2002) and similar USEPA (1999) methods. Samples will be collected in both riffle/run and pool habitats. The riffle community will likely have the greatest diversity and extends about 1,000 feet downstream of the plunge pool immediately below Burt Dam. This stretch of the creek was the focus of stream bank and stream channel restoration efforts in 2002 and 2003 and is, therefore, a likely area that would be indicative of ecological recovery. Table 1-9 provides an overview of the planned sampling and analysis activities.

E & E intends to conduct the field work in August of 2012. Two samples of benthic macroinvertebrates in the riffle/run habitat will be collected using the "traveling kick" method. This method is appropriate for sampling shallow riffle/run habitat where biological diversity is at a maximum and any pollution-sensitive macroinvertebrates would occur. However, the macroinvertebrates of the riffle/run community live on and in the interstitial cavities between rocks, where they are not directly exposed to sediments. Consequently, sediment samples for chemical analysis and toxicity testing will not be collected at the two riffle/run locations where benthic macroinvertebrates are collected.

Table 1-9 Summary of Eighteenmile Creek Benthic-Community Sampling Project

		Parameter		
Sample	Habitat Type	Benthic Community Composition[a]	Sediment Chemistry[b]	Sediment Toxicity[c]
1	Riffle	X		
2	Riffle	X		
3	Pool	X	X	X
4	Pool	X	X	X
5	Pool	X	X	X

Notes:
[a] Macroinvertebate abundance and diversity and metrics.
[b] PCB Aroclors and congeners, TAL inorganic analytes, AVS/SEM, TOC, grain size, and density.
[c] 10-day sediment bioassays with *Hyalella azteca* (amphipod) and *Chironimus dilutus*(midge).

Key:
TAL = target analyte list
TOC = total organic carbon

In addition, E & E will collect benthic macroinvertebrate samples at three locations with homogenous soft sediments in the AOC downstream of the riffle/run area. The soft sediments will be collected with a petite Ponar or Ekman dredge from a boat and the organisms will be separated from the sediment by screening in the laboratory. At these locations, sediment also

will be collected for chemical analysis (PCB Aroclors and congeners, metals, acid volatile sulfides/simultaneously extracted metals (AVS/SEM), TOC, grain size, and density) and toxicity testing (10-day survival and growth tests with *Hyalella azteca* and *Chironimus dilutus*).

In addition to the collecting macroinvertebrates and sediments, physical and habitat parameters will be measured or noted in accordance with NYSDEC (Bode et al. 2002) and USEPA (1999) methods.

Benthic macroinvertebrate samples will be preserved with ethyl alcohol solution and shipped to REI Consultants, Inc. (REIC) for macroinvertebrate identification and enumeration. Sediment samples will be transmitted to ALS-Kelso for chemical analysis and to Aquatec Biological Sciences for toxicity testing, as described in Section 2.3. Analytical methods and quality objectives and criteria are presented in Section 2.4.

1.3.4 General Subtasks Common to All Three Projects

The three projects described above can each be broken down into four general subtasks: (1) preparation of planning documents; (2) field sampling; (3) laboratory analysis; and (4) report preparation. This QAPP represents subtask 1 for all three projects. Subtasks 2 to 4 will be implemented as soon as possible after QAPP approval (see Section 1.3.5 for detailed schedule).

The end users of the data from the three investigations described in this QAPP will be the NCSWCD, USACE, USEPA, NYSDEC, NYSDOH, and other groups with an interest in remediating and eventually delisting the Eighteenmile Creek AOC. Data collected for these investigations will be evaluated relative to the standards and criteria presented in Section 1.4

1.3.5 Project Schedule

A detailed schedule for implementing the three projects described in this QAPP is provided in Table 1-10. Field sampling will need to occur in late July or early August 2012 in order for the final report to be completed before the end of 2012.

Table 1-10 Schedule for Eighteenmile Creek PAC Pilot Study and Baseline Fish and Benthic-Community Sampling

Month and Day	Duration	Activity
May 9 - 25	2.5 weeks	Prepare draft QAPP
May 28 - June 22	4 weeks	USEPA review of draft QAPP
June 25 - July 6	2 weeks	Revision of draft QAPP based on USEPA comments
July 9 - 20	2 weeks	EPA review and approval of revised QAPP
July 23 - Aug. 10	3 weeks	Field-work time window
Aug. 13 - Oct. 19	10 weeks	Laboratory testing and analysis and data validation
Oct. 22 - Nov. 9	3 weeks	Prepare draft report
Nov. 12 - Dec. 7	4 weeks	EPA review of draft report
Dec. 10 - Dec. 21	1 week	Revise draft report based on USEPA comments

Key:
QAPP = quality assurance project plan
USEPA = United States Environmental Protection Agency

1.4 Quality Objectives and Criteria

Data quality objectives (DQOs) are qualitative and quantitative statements that clearly define the objectives of the project, define the most appropriate types of data, determine the appropriate procedures for data collection, and specify acceptable decision error limits that establish the quantity and quality of data needed for decision making. The specific objectives of this project are provided in Sections 1.3.1 to 1.3.3. The general data quality objectives for each project data collection activity summarized in Table 1-11. Acceptance and performance criteria for field and analytical QC samples are outlined in Section 2. Appendix B of this QAPP provides detailed acceptance and performance criteria for analytical methods in the laboratory SOPs.

Acceptance and performance criteria are often specified in terms of precision, accuracy, representativeness, completeness, and comparability (PARCC) parameters. Numerical acceptance criteria cannot be assigned to all PARCC parameters, but general performance goals are established for most data collection activities. Data assessment procedures throughout the QAPP clearly outline the steps to be taken, responsible individuals, and implications if QA objectives are not met. PARCC parameters are briefly defined below.

1.4.1 Precision

Precision measures the reproducibility of measurements under a given set of conditions. Specifically, it is a quantitative measure of the variability of a group of measurements compared to their average value, usually stated in terms of standard deviation or coefficient of variation. It also may be measured as the relative percent difference (RPD) between two values. Precision includes the interrelated concepts of instrument or method detection limits and multiple field sample variance. Sources of this variance are sample heterogeneity, sampling error, and analytical error.

1.4.2 Accuracy

Accuracy measures the bias of the measurement system. Sources of this error are the sampling process, field contamination, preservation, handling, sample matrix, sample preparation, and analysis. Data interpretation and reporting may also be significant sources of error. Typically, analytical accuracy is assessed through the analysis of spiked samples and may be stated in terms of percent recovery or the average (arithmetic mean) of the percent recovery. Blank samples are also analyzed to assess sampling and analytical bias (i.e., sample contamination). Background measurements similarly assess measurement bias. The number of samples collected will impact the confidence of the statistical data evaluation.

Table 1-11 General Data Quality Objectives

Data Collection Activity	Quality Objectives	Standards[a]	Acceptability/ Performance Criteria[b]
Historical Data Collection	To incorporate relevant existing data that meets quality objectives for the project. Historic sediment sampling data must be geo-referenced.	■ EPA or NYSDEC sampling and analytical procedures	■ Data are generated using EPA or NYSDEC sampling and analytical methods or alternative methods that meet project objectives. ■ Data must be from the original source. All historical sediment chemistry data was previously evaluated under the Remedial Investigation and the database will be used without further evaluation. ■ Data must be geo-referenced or able to be digitized into a GIS system.
Sampling and Analysis	To have samples and analytical results that accurately represents the nature and extent of contamination at the site. Data must be of sufficient quality to meet regulatory requirements and allow assessment of impacts on human health and the environment by comparison to New York State criteria or background values. Data also may be used to assess long-term trends, re-evaluate selected BUIs, and re-evaluate fish consumption advisories.	■ NYSDEC freshwater sediment standards and fish flesh criteria. ■ Applicable freshwater sediment screening levels, such as those from MacDonald et al. (1999).	■ Data must be collected under an approved QAPP using approved SOPs. Data must meet the acceptance and performance criteria documented in Section 2 of this QAPP. ■ Reporting limits should be below risk-based screening values for 90% of target analytes and 100% of critical analytes of concern. ■ Data must be comparable to applicable standards.
Documenting Sampling Locations	To relate project sample locations to existing local benchmarks.	■ Composite sediment sample areas will be located and recorded on detailed aerial photographs.	■ Relation of sampling locations to existing/known benchmarks.
Field Records	To document all field activities and to allow accurate representation field events in the final report. Records must be capable of withstanding legal scrutiny.	■ Section 2 of the QAPP ■ E & E SOPs (Field Activities Logbooks)	■ Consistency between field and laboratory data. ■ Clear and legible documentation for sample collection and equipment decontamination for final report.

Table 1-11 General Data Quality Objectives

Data Collection Activity	Quality Objectives	Standards[a]	Acceptability/ Performance Criteria[b]
Outside Records	To use the most current reference values, reports, or data from outside sources in data assessments and recommendations for the site.	■ None	■ All versions of data or standards must be the most current values available. ■ Data or standards must be accurately incorporated into the final report.
Data Review and Assessment	To review and verify data are generated according to the QAPP, and assign data qualifiers as necessary to indicate limitations on data usability.	■ NYSDEC DUSR Guidance ■ EPA Region 2 Data Validation SOPs ■ EPA National Functional Guidelines	■ Data must be reviewed by Project Chemist meeting minimum NYSDEC qualifications. ■ Data qualifiers or changes to data must be documented in a DUSR.

Notes:
[a] Major standards.
[b] Major or noteworthy acceptability criteria. All performance criteria must be verified using procedures listed in the QAPP.

Key:
BUI = Beneficial Use Impairment
DUSR = Data Usability Summary Report
GPS = Global Positioning System
NYSDEC = New York State Department of Environmental Conservation
NYSDOH = New York State Department of Health
SOP = Standard Operating Procedure
QAPP = Quality Assurance Project Plan

1.4.3 Representativeness

Representativeness expresses the degree to which data represent a characteristic of a population, a parameter variation at a sampling point, or an environmental condition. Representativeness is a qualitative parameter, which is most concerned with proper design of the measurement program. Sample/measurement locations may be biased (judgmental) or unbiased (random or systematic). Representativeness of the sampling scheme will be determined with evaluation of the historical data and statistical evaluation of the results compared to the reference site or existing data.

1.4.4 Completeness

Completeness is defined as the percentage of measurements performed which are judged to be valid. Although a quantitative goal must be specified, the completeness goal is the same for all data uses—that a sufficient amount of valid data should be generated. A completeness goal of 90% is established for this project.

1.4.5 Comparability

Comparability is a qualitative parameter expressing the confidence with which one data set may be compared to another. Sample data should be comparable with other measurement data for similar samples and sample conditions. This goal is achieved through the use of standard techniques to collect and analyze samples. Historical data will be evaluated to ensure the methods and reporting limits are comparable to the proposed sampling. Data will only be evaluated if it is determined to be comparable. For this investigation, PCB Aroclors and congeners in biota and sediment are being analyzed using EPA Method 8082 because previous investigations into bioaccumulation of PCBs by fish and benthic organisms (USACE 2004a and 2004b, von Stackelberg and Gustavson 2012) used this analytical method.

1.5 Special Training/Certification

In general, personnel responsible for performing technical tasks and evaluating project data will have the appropriate education background, experience, and certifications (if applicable) to ensure that they possess the degree of specialization and technical competence necessary to perform the required work effectively and efficiently. Specific training requirements for project activities are outlined in Table 1-12.

Table 1-12 Project Special Training and Certification Requirements

Affiliation and Role	Required Training or Certifications
Field Staff	■ 40-hour health and safety training ■ CPR/first aid certification course ■ Eight hours of refresher training annually
GIS Analyst	■ Extensive experience with ESRI tools – especially ArcGIS Desktop 10.x ■ Understanding of principles of cartography and effective map design ■ Specific ArcGIS experience with geoprocessing tools and data analysis, including Raster analysis with Spatial Analyst ■ Working knowledge of EQuIS or equivalent chemical RDBMS for collaboration with the project chemist ■ EPA data quality standards ■ Relational database design
Project Chemist	■ DOT hazard waste shipping ■ Equis database operation
Subcontractors	
Laboratory for sediment and tissue chemical analysis	Certified by National Environmental Laboratory Accreditation Program (NELAC) standards

Key:
CPR = cardiopulmonary resuscitation
DOT = (United States) Department of Transportation
EPA = (United States) Environmental Protection Agency
NELAC = National Environmental Laboratory Accreditation Program
RDBMS = Relational Database Management System

1.6 Documentation and Records

E & E project records will be maintained on internal secure project drives that are accessible only to the project team. Project documents will be delivered to the NCSWCD as portable document format (PDF) files or posted on a secure file transfer protocol (FTP) site. Field records will be generated in hard copy field logs or on project-specific datasheets as specified in E & E's Standard Operating Procedures (SOPs) for field activities logbooks. Details are provided in Table 1-13.

Table 1-13 Project Record Requirements

Data Type	Data Form/Location	Retrieval Form	Length of Retention by E & E	Report Submittal Frequency/ Type	Originator	Recipient
Project Deliverables	Publications Secure Server Driver	Released by Editor	5 years	Posted on a secure FTP site	E & E	NCSWCD
Field Logbooks and Forms	Hard Copy/ Project Manager Files	PM has access	5 years	None	Field Personnel	Project Manager
	Electronic (PDF)/ internal secure project drive	Project team has access	5 years	Appendix to Technical Report	E & E	NCSWCD
Field Data	Electronic file from GPS/ internal secure project drive	GIS Analyst	5 years	EQuis	E & E	NCSWCD
Laboratory Reports	Electronic (PDF)/ internal secure project drive	SUBLAB System	5 years	Appendix to Technical Report	Laboratory	Project Chemist
Laboratory EDD	Electronic spreadsheet or CSV/ internal secure project drive	SUBLAB System	5 years	EQuis	Laboratory	Project Chemist and NCSWCD
Historical Data	Electronic spreadsheet or CSV/internal secure project drive	Project team has access	Data will be incorporated in report.	EQuis	E & E	NCSWCD
Modeling or Data Calculations	Electronic spreadsheet or CSV/ internal secure project drive	Project team has access	Data will be incorporated in report.	Technical Report	E & E	NCSWCD

Key:

CSV = comma delimited file (file extension for a comma delimited file)
EDD = electronic data deliverable
GIS = geographic information system
NCSWCD = Niagara County Soil and Water Conservation District
PDF = portable document format

1.6.1 Record Retention

Project-related records will be stored in secure areas consistent with requirements of the contract. E & E will transfer project records to the client for storage with the final project files.

1.6.2 Field Documentation

Field records include the following:

- A log of daily activities and visual observations of the project area on data collection forms or in a field notebook (including weather conditions, personnel present, site entry and departure time, and time and location of samples);
- Field measurements and equipment used (including equipment calibration records if applicable);
- Daily activities summary form;
- Health and safety log;
- Data collection forms (see Appendix C); and
- Photographs of sample locations or significant features noted on the data collection forms or field notebooks. The following information will be noted in the field notebook concerning photographs:
  - Date, time, location, and direction photograph was taken;
  - Description of the photograph taken;
  - Sequential number of the digital photo; and
  - Camera system used.

1.6.3 Laboratory Reports

Laboratory reports will be consistent with NYSDEC Analytical Services Protocol Level B requirements and/equivalent requirements. Electronic data will be provided consistent with NYSDEC Equis electronic data deliverable (EDD) format. All other laboratory records will be maintained by the laboratory in accordance with the certified procedures.

1.6.4 Project Report

The project report is expected to include the following:

- Executive summary;
- Project background (e.g., goals and objectives, general regional area description, site history, relevance to BUI delisting, relevance to upcoming FS and remedial work);
- Field investigation activities and methodology (figures showing sampling locations);

- Deviations from planned activities;
- Results (discussion and tabulated results); and
- Conclusions and recommendations.

The appendices may include:

- Electronic data;
- Laboratory reports;
- Data review memos; and
- Calculations (if applicable).

1.6.5 QAPP Revision and Distribution

The E & E QA Officer will be responsible for updating the QAPP and distributing the revised QAPP to the personnel listed in the distribution list.

2 Data Generation and Acquisition

This section of the QAPP contains descriptions of all aspects of the implementation of data generation and acquisition procedures or project processes. Field, laboratory and data handling procedures were designed to meet applicable NYSDEC and USEPA requirements.

2.1 Project or Sampling Design

As described in Section 1.3, three investigations are described in this QAPP: (1) pilot study on the use of PAC to reduce PCB bioavailability in Eighteenmile Creek sediment; (2) Eighteenmile Creek baseline fish sampling; and (3) Eighteenmile Creek baseline benthic-community sampling. The sampling process design was presented in Section 1.3.1 and Table 1-3 for the PAC pilot study project; in Section 1.3.2 and Tables 1-5 and 1-6 for the baseline fish sampling project; and in Section 1.3.3 and Table 1-7 for the baseline benthic-community sampling project.

2.2 Sampling Methods

For routine procedures related to sediment and fish sampling, sample packaging and shipping, equipment contamination, field logbook entries, and disposal of investigation derived waste, E & E SOPs will be followed. The following E & E SOPs are included in Appendix A to guide these activities:

FIELD ACTIVITY LOGBOOKS	DOC 2.1
AQUATIC SEDIMENT SAMPLING	ENV 3.8
SAMPLING EQUIPMENT DECONTAMINATION	ENV 3.15
SAMPLE PACKAGING AND SHIPPING	ENV 3.16
PROCEDURE FOR HANDLING INVESTIGATION-DERIVED WASTES	ENV 3.26
PROCEDURE FOR FISH SAMPLE COLLECTION	ENV 3.28

The following sections provide an overview of field sampling activities for the three investigations described in this QAPP.

2.2.1 PAC Pilot Study Sediment Sampling Methodology

Sediment sampling areas for the PAC pilot study are listed in Table 1-5 and shown in Figures 1-4 to 1-6. One sampling area was selected in each of the three main reaches of the creek: (1) below Burt Dam; (2) between Newfane and Burt dams; and (3) upstream from Newfane Dam. At each area, approximately 10 gallons of sediment will be collected by taking multiple hauls with an Ekman dredge across the sampling area. After retrieval, the top of the dredge will be opened and surface sediment (0 to 6 inches below the sediment water interface) will be removed with a stainless-steel spoon or Teflon scope and placed into sampling buckets. If there is a thin layer of water at the top within the dredge, it will be removed with small hand-powered suction device (such as a turkey baster). Because bulk sediment (porewater plus the solids) is used in the bioaccumulation tests this effort is to remove only overlying water as the intent is not to remove porewater from sediment solids.

Three 3.5-gallon pre-cleaned plastic buckets will be filled at each sample area. Surface sediment only will be included in the samples. Between sampling areas, the dredge will be thoroughly cleaned with an Alconox detergent solution and thoroughly rinsed. Samples will be cooled to 4°C and shipped in coolers under chain-of-custody (COC) by overnight courier to Aquatec Biological Sciences for bioaccumulation testing. Further mixing of the sediment in the three buckets from each sampling area to create a single composite sample for each area will be conducted at Aquatec. Aquatec will send subsamples of each composite sample to ALS-Kelso for chemical analysis. Table 2-1 lists analytical parameters and methods and sample-handling details for the PAC pilot study. All analytical parameters listed in Table 2-1 for the PAC pilot study are considered critical to the success of the study.

2.2.2 Baseline Fish and Sampling Methodology

Brown bullhead, pumpkinseed composite samples, and crayfish composite samples will be collected from each of the three main reaches of the creek: (1) below Burt Dam; (2) between Newfane and Burt dams; and (3) upstream from Newfane Dam. In addition, largemouth bass will be collected from the reach upstream from Newfane Dam. Numbers of samples per reach are listed in Table 1-7 and additional sample details are provided in Table 1-6. If the target species are not available, alternate species will be collected (see Table 1-6). The fish and crayfish will be collected from where they are available within each reach at the time of sampling (late July or August 2012). Collection locations will be recorded in the field with a hand-held Garmin or similar Geographic Positioning System (GPS) unit and noted with respect to fixed landmarks. A collection permit will be acquired from NYSDEC prior to initiation of the work.

The fish will be collected by electroshocking and seining by a three-person field crew. Crayfish will be collected with standard Gee crayfish traps baited with fish from Eighteenmile Creek and deployed overnight at multiple locations in each reach. Brown bullhead and largemouth bass will be weighted to the nearest gram and the total length measured to the nearest millimeter. Pumpkinseeds to be included in composite samples also will be weighted and measured and the number of fish in each composite sample will be recorded. Crayfish to be included in composite samples also will be weighed and measured and the number of crayfish in each composite

sample will be recorded. For the pumpkinseed and crayfish composite samples, the target number of individuals per sample is 10 (see Table 1-6). After collection, each fish and crayfish sample will be wrapped in aluminum foil, placed in Ziploc or similar plastic bag, placed in a cooler on ice, and clearly labeled. The fish and crayfish samples will be frozen if logistics will not permit the samples to arrive at the analytical laboratory (ALS-Kelso) within 24 hours of collection. Processing of fish and crayfish samples will take place at ALS-Kelso following NYSDEC (2008) protocols. Processing will included removing skin-off fillets from bullheads and homogenization of whole-body largemouth bass samples and whole-body composite samples of pumpkinseeds and crayfish. Table 2-1 summarizes analytical parameters and methods and sample-handling details for the baseline fish sampling study. All analytical parameters listed in Table 2-1 for this study are considered critical to the study's success.

2.2.3 Baseline Benthic Community Sampling Methodology

Sediment and benthic macroinvertebrate samples will be collected for this project (see Section 1.3.3). Sampling will occur in late July or early August 2012. All samples will be collected from the creek below Burt Dam. Sediment will be collected from three pool locations and benthic macroinvertebrates will be collected from two riffle locations and three pool locations (see Table 1-9). Sampling locations will be recorded in the field with a hand-held Garmin or similar GPS unit and noted with respect to fixed landmarks. A permit for collecting benthic macroinvertebrates will be acquired from NYSDEC prior to initiation of the work. Benthic macroinvertebrate sampling will be consistent with NYSDEC (Bode et al. 2002) and USEPA (1999) protocols. Macroinvertebrate samples will be shipped under COC to REI Consultants for identification and enumeration. Sediment samples will be shipped under COC by overnight courier to ALS-Kelso for chemical analysis.

Sediment will be collected from pool locations with an Ekman dredge from a small boat. After retrieval, the top of the dredge will be opened and surface sediment (0 to 6 inches below the sediment water interface) will be removed with a stainless-steel or Teflon scope, placed into a large stainless-steel bowl, homogenized, and placed into sample containers. Large pieces of organic matter (i.e., sticks and leaves), stones, and other debris will be removed from the sediment samples. Table 2-1 lists the parameters that will be measured in sediment, appropriate sample containers, preservation methods, and holding times. At one of the three pool sample locations, a duplicate sample will be collected for the sediment chemical parameters listed on Table 2-1. A duplicate sample will not be taken for sediment toxicity because replication is built into the USEPA (2000) testing protocol; each sample is divided into five replicates and each replicate is tested individually. Table 2-1 summarizes analytical parameters and methods and sample-handling details for the baseline benthic community sampling study. All analytical parameters listed in Table 2-1 for this study are considered critical to the study's success.

Table 2-1 Analytical Methods, Sample Containers, Preservatives, and Holding Times for Biota and Sediment Sampling at the Eighteenmile Creek, Niagara County, New York

Sample Type	Preparation/Analysis	Method	Number of Samples	Sample Container	Preservation	Maximum Holding Time
Baseline Fish Sampling Project						
Fish[a]	PCB Congeners and Aroclors (EPA 8082 list)	EPA 8082	44	Biota samples will be tagged, wrapped in aluminum foil, placed in a Ziploc or similar plastic bag, and placed in a cooler on ice.	4°C or frozen if receipt at lab will be > 24 hrs after collection.	365 days to extraction; 35 days from extraction to analysis
	Percent Lipids	See note d.	44			See note d.
	Percent Moisture	See note d.	44			See note d.
Baseline Benthic Community Assessment Project						
Sediment[b]	Total Organic Carbon	See note d.	4	Amber 4-oz glass jar with Teflon-lined cap.	4°C	28 days
	Grain Size Distribution (percent sand, silt, clay)	ASTM D422	4	Amber 8-oz glass jar with Teflon-lined cap.	4°C	28 days
	PCB Congeners and Aroclors (8082 list) and chlorinated pesticides	EPA 8082 and 8081B	4	Amber 8-oz glass jar with Teflon-lined cap.	4°C	14 days to extraction; 35 days from extraction to analysis
	Density, wet	ASTM D854	4	Amber 4-oz glass jar with Teflon-lined cap.	4°C	na
	Total Metals (TAL list)	EPA 200.8, 6010B, 7471A	4	Amber 4-oz glass jar with Teflon-lined cap.	4°C	180 days
	AVS/SEM (Cd, Cu, Ni, Pb, Hg, Zn, Ag)	EPA (1991) draft method for AVS/SEM in sediment and EPA	4	Amber 4-oz glass jar filled to the brim with no air space	4°C	14 days for AVS

Table 2-1 Analytical Methods, Sample Containers, Preservatives, and Holding Times for Biota and Sediment Sampling at the Eighteenmile Creek, Niagara County, New York

Sample Type	Preparation/Analysis	Method	Number of Samples	Sample Container	Preservation	Maximum Holding Time
		6010, 6020, and 7471 for metals.				
Sediment Toxicity	Toxicity - *Hyalella azteca* (10-day)	EPA 100.1	3	1-gal Ziploc bag (double bagged)	4°C	8 weeks
	Toxicity - *Chironomus dilutus* (10-day)	EPA 100.2	3	1-gal Ziploc bag (double bagged)	4°C	8 weeks
Benthic Macroinvertebrates	Identification and enumeration–riffle/run community	See note d.	2	4-oz glass jar with Teflon-lined cap	70 to 95% ethanol	See note d below.
	Identification and enumeration–pool community	See note d.	2	2 x 1-gallon plastic jar per location	70% or 95% ethanol to result in a 10% ethanol solution	See note d below.
PAC Bioaccumulation Reduction Pilot Study Project						
Bioaccumulation Testing[c]	4-day screening test with *Lumbriculus variegatus*	EPA 100.3	3	Three pre-cleaned 3.5-gallon plastic buckets from each of three (3) sample sites.	4°C	8 weeks
	Prepare PAC treatments for 28-day bioaccumulation tests	na	12			
	28-day bioaccumulation test with *Lumbriculus variegatus*	EPA 100.3	12			
Lumbriculus tissue	Lipids	See note d.	12	Amber 4-oz glass jar	4°C	14 days to extraction; 35 days from extraction to analysis

Table 2-1 Analytical Methods, Sample Containers, Preservatives, and Holding Times for Biota and Sediment Sampling at the Eighteenmile Creek, Niagara County, New York

Sample Type	Preparation/Analysis	Method	Number of Samples	Sample Container	Preservation	Maximum Holding Time
	PCB Congeners and Aroclors (8082 list)	EPA 8082	12	Amber 4-oz glass jar	4°C	14 days to extraction; 35 days from extraction to analysis
Sediment	Total Organic Carbon	See note d.	12	Amber 4-oz glass jar	4°C	28 days
	PCB Congeners and Aroclors (8082 list)	EPA 8082	12	Amber 4-oz glass jar	4°C	14 days to extraction; 35 days from extraction to analysis
	Percent Solids	ASTM D2216	12	Amber 4-oz glass jar	4°C	na

Notes:
[a] 20 gamefish (15 bullhead + 5 largemouth bass) skin-on fillet samples, 15 forage fish composite samples, and nine crayfish composite samples.
[b] Three original samples and one field duplicate.
[c] Four (4) PAC treatments (5%, 3%, 1%, and 0% [control]) will be prepared from each of the three (3) sediment samples. See Section 1.3.1 for further detail.
[d] Laboratory SOP provided in Appendix B.

Key:
AOC = Area of Concern
ASTM = American Society of Testing and Materials
AVS = acid volatile sulfide
Na = not applicable
PAC = powdered activated carbon
PCB = polychlorinated biphenyls
SEM = simultaneously extracted metals
TAL = target analyte list

Benthic macroinvertebrate samples for identification and enumeration will be collected from both riffle and pool habitats. Riffle habitat is present only for the first 1,000 feet downstream from Burt Dam. The selection of specific locations for riffle community sampling will be made in the field by an experienced biologist. Sites will be selected that are expected to have high diversity of macroinvertebrates, within the constraints posed by access and safety considerations. The most diverse communities typically are present in areas with mixes of sand, gravel, and cobble substrates with perennial flows over the top of the substrate. Ideal flow rates are 0.3 to 1 meter per second (m/s). Safety considerations would preclude sites that require wading through water above hip-wader depth or that are flowing faster than 1 m/s. Riffles community samples will be collected using the "traveling kick" method. This method involves using a handheld rectangular net with 500-micrometer mesh that is held with the mouth of the net upstream. The substrate upstream of the net is kicked up and resident macroinvertebrates are carried by the current into the net. The net is then emptied into a clean white pan and the macroinvertebrates are picked out of the pan. Samples will be preserved with 70% or 95% ethyl alcohol.

E & E also will collect benthic macroinvertebrate samples from two locations with homogenous, soft sediments in the AOC downstream of the riffle area. These locations will coincide with two locations where samples are collected for sediment chemistry and toxicity testing. The soft sediments will be collected with a petite Ponar or Ekman dredge from a boat and the organisms will be separated from the sediment by screening in the laboratory. The raw sediment samples will be transferred from the dredge into wide-mouth plastic gallon jars with sufficient 70% or 95% ethyl alcohol to result in a 10% ethyl alcohol solution after being filled with sediment.

Finally, at each macroinvertebrate sampling location, the following parameters will be measured or noted in accordance with NYSDEC (Bode et al. 2002) and USEPA (1999) protocols.

- Sampling site (station number and specific location);

- Collection date and time (arrival and departure) and names of collectors;

- Physical parameters:
  - Water depth,
  - Stream width,
  - Current speed,
  - Substrate type,
  - Bank stability,
  - Canopy cover, and
  - Embeddedness; and

- Water quality parameters:
  - Temperature,
  - Dissolved oxygen,
  - pH,
  - Specific conductance, and
  - Aquatic vegetation types and approximate coverage.

2.3 Sample Handling and Custody

2.3.1 Sample Containers, Preservation, and Holding Times

The volumes and containers and holding times required for sampling activities are indicated in Table 2-1. Prewashed sample containers will be provided by the laboratory and will be wide-mouth jars with Teflon-lined caps unless otherwise indicated. The laboratory must use an approved specialty container supplier, which prepares containers in accordance with EPA bottle-washing procedures. The laboratory must maintain a record of all sample bottle lot numbers shipped in the event of a contamination problem. As indicated in Table 2-1, the sample preservation methods required for the investigations described herein include cooling sediments to 4 degrees Celsius (°C) for chemical analysis, bioaccumulation testing, and toxicity testing; cooling to 4°C or freezing for fish and crayfish for chemical analysis; and addition of ethyl alcohol to benthic macroinvertebrate samples.

2.3.2 Sample Handling

The transportation and handling of samples must be accomplished in a manner that not only protects the integrity of samples but also prevents any detrimental effects due to the possible hazardous nature of the samples. Regulations for packaging, marking, labeling, and shipping of hazardous materials are promulgated by the United States Department of Transportation (DOT) in 49 CFR 171 through 177. E & E trains all staff responsible for the shipment of samples in these regulations. Procedures for sample packing and shipping are documented in E & E SOP ENV 3.16 (see Appendix A).

2.3.3 Sample Custody

Formal sample custody procedures begin when pre-cleaned sample containers leave the laboratory or upon receipt from the container vendor. The laboratory must follow written and approved SOPs for shipping, receiving, logging, and internally transferring samples. Sample identification documents must be carefully prepared so that sample identification and COC can be maintained and sample disposition controlled.

The primary objective of COC procedures is to provide an accurate written or computerized record that can be used to trace the possession and handling of a sample from sampling through completion of all required analyses. A sample is in custody if it is:

- In a team member's physical possession;
- In a team member's view;
- Secured in a locked container of some sort; or
- Kept in a secured area where access is restricted to authorized personnel only.

The COC form must be fully completed by the field technician designated by the Project Manager as responsible for sample shipment to the appropriate laboratory for analysis.

2.4 Analytical Method Requirements

Analytical method requirements are listed in Tables 2-1 and 2-2. Project action limits and method detection limits are provided on Table 2-2. The specific implementation of analytical methods is documented in laboratory SOPs provided in Appendix B.

2.5 Quality Control

QC data are necessary to determine precision and accuracy and to demonstrate the absence of interferences and/or contamination of glassware and reagents. Field and laboratory QC samples are summarized on Tables 2-3 and 2-4, respectively. Method QC limits for analyses are provided in Table 2-4 and Appendix B.

2.6 Instrument/Equipment Testing, Inspection, and Maintenance

All laboratory and field instruments and equipment used for sample analysis must be serviced and maintained only by qualified personnel. Laboratory instrument maintenance procedures will be evaluated to verify that there will be no impacts on analysis of project samples due to instrument malfunction. For example, the laboratory must have duplicate instrumentation and/or maintained major equipment under service agreements with the manufacturer that require rapid respond by manufacturer approved service agents.

Field instruments will be rented through approved suppliers that have manufacturer-approved maintenance programs. Field equipment will be checked upon receipt to verify that instruments are in good working condition and that the rental company provided appropriate calibration records or certifications. On-site operation will be performed in accordance with manufacturer manuals. If any problems occur, the instrument will be replaced immediately.

2.7 Instrument/Equipment Calibration and Frequency

All instruments and equipment used during sampling and analysis will be operated and calibrated according to the manufacturer's guidelines and recommendations, as well as criteria set forth in applicable analytical methodology references. Personnel properly trained in these procedures will perform operation and calibration of all instruments. Documentation of all field maintenance and calibration information will be maintained in the field logbook. Table 2-5 lists typical monitoring equipment used during fieldwork. The measurements listed in this table are for informational purposes only.

Table 2-2 Analytical Methods and Performance Criteria for Analysis of Sediment and Biological Tissue Samples for Eighteenmile Creek, Niagara County, New York

Sample Type	Analysis Description	Method	Analyte	Units	Method Detection Limit	Method Reporting Limit	Screening Level	
							Value	Source
Baseline Fish Sampling								
Fish and Crayfish[a]	PCB Congeners and Aroclors (EPA 8082 list)	EPA 8082	Aroclor 1016	µg/kg wet	2.4	10	440	Dyer et al. 2000
			Aroclor 1221	µg/kg wet	2.4	20	440	Dyer et al. 2000
			Aroclor 1232	µg/kg wet	2.4	10	440	Dyer et al. 2000
			Aroclor 1242	µg/kg wet	2.4	10	440	Dyer et al. 2000
			Aroclor 1248	µg/kg wet	2.4	10	440	Dyer et al. 2000
			Aroclor 1254	µg/kg wet	2.4	10	440	Dyer et al. 2000
			Aroclor 1260	µg/kg wet	2.4	10	440	Dyer et al. 2000
			Congener IUPAC # 1	µg/kg wet	1.5	5	440	Dyer et al. 2000
			Congener IUPAC # 5	µg/kg wet	0.1	0.5	440	Dyer et al. 2000
			Congener IUPAC # 18	µg/kg wet	0.1	0.5	440	Dyer et al. 2000
			Congener IUPAC # 31	µg/kg wet	0.1	0.5	440	Dyer et al. 2000
			Congener IUPAC # 44	µg/kg wet	0.3	0.5	440	Dyer et al. 2000
			Congener IUPAC # 52	µg/kg wet	0.4	0.5	440	Dyer et al. 2000
			Congener IUPAC # 66	µg/kg wet	0.6	1	440	Dyer et al. 2000
			Congener IUPAC # 87	µg/kg wet	0.2	0.5	440	Dyer et al. 2000
			Congener IUPAC # 101	µg/kg wet	0.4	0.5	440	Dyer et al. 2000
			Congener IUPAC # 110	µg/kg wet	0.3	0.5	440	Dyer et al. 2000
			Congener IUPAC # 138	µg/kg wet	0.09	0.5	440	Dyer et al. 2000
			Congener IUPAC # 141	µg/kg wet	0.5	0.5	440	Dyer et al. 2000
			Congener IUPAC # 151	µg/kg wet	0.09	0.5	440	Dyer et al. 2000
			Congener IUPAC # 153	µg/kg wet	0.1	0.5	440	Dyer et al. 2000
			Congener IUPAC # 170	µg/kg wet	0.4	0.5	440	Dyer et al. 2000
			Congener IUPAC # 180	µg/kg wet	0.3	0.5	440	Dyer et al. 2000
			Congener IUPAC # 183	µg/kg wet	0.2	0.5	440	Dyer et al. 2000
			Congener IUPAC # 187	µg/kg wet	0.08	0.5	440	Dyer et al. 2000
			Congener IUPAC # 206	µg/kg wet	0.2	0.5	440	Dyer et al. 2000
	Percent lipids	See note d.	Lipid	% wet wt.	na	0.1	0.2	na
	Percent moisture	See note d.	Moisture content	% wet wt.	na	0.1	na	na

Table 2-2 Analytical Methods and Performance Criteria for Analysis of Sediment and Biological Tissue Samples for Eighteenmile Creek, Niagara County, New York

Sample Type	Analysis Description	Method	Analyte	Units	Method Detection Limit	Method Reporting Limit	Screening Level	
							Value	Source
Baseline Benthic Community Assessment								
Sediment[b]	Total organic carbon	See note d.	Total organic carbon	% dry wt.	0.04	0.1	1	Persaud et al. 1993
	Grain size (% sand, silt, clay)	ASTM D422	% sand, silt, clay	% dry wt.	NA	0.1	1	na
	PCB Congeners and Aroclors (8082 list)	EPA 8082	Aroclor 1016	µg/kg dry	2.1	10	60	MacDonald et al. 2000
			Aroclor 1221	µg/kg dry	2.1	20	60	MacDonald et al. 2000
			Aroclor 1232	µg/kg dry	2.1	10	60	MacDonald et al. 2000
			Aroclor 1242	µg/kg dry	2.1	10	60	MacDonald et al. 2000
			Aroclor 1248	µg/kg dry	2.1	10	60	MacDonald et al. 2000
			Aroclor 1254	µg/kg dry	2.1	10	60	MacDonald et al. 2000
			Aroclor 1260	µg/kg dry	2.1	10	60	MacDonald et al. 2000
			Congener IUPAC # 1	µg/kg dry	1.2	5	60	MacDonald et al. 2000
			Congener IUPAC # 5	µg/kg dry	0.2	0.5	60	MacDonald et al. 2000
			Congener IUPAC # 18	µg/kg dry	0.09	0.5	60	MacDonald et al. 2000
			Congener IUPAC # 31	µg/kg dry	0.07	0.5	60	MacDonald et al. 2000
			Congener IUPAC # 44	µg/kg dry	0.3	0.5	60	MacDonald et al. 2000
			Congener IUPAC # 52	µg/kg dry	0.2	0.5	60	MacDonald et al. 2000
			Congener IUPAC # 66	µg/kg dry	0.1	1	60	MacDonald et al. 2000
			Congener IUPAC # 87	µg/kg dry	0.2	0.5	60	MacDonald et al. 2000
			Congener IUPAC # 101	µg/kg dry	0.08	0.5	60	MacDonald et al. 2000
			Congener IUPAC # 110	µg/kg dry	0.08	0.5	60	MacDonald et al. 2000
			Congener IUPAC # 138	µg/kg dry	0.08	0.5	60	MacDonald et al. 2000
			Congener IUPAC # 141	µg/kg dry	0.06	0.5	60	MacDonald et al. 2000
			Congener IUPAC # 151	µg/kg dry	0.07	0.5	60	MacDonald et al. 2000
			Congener IUPAC # 153	µg/kg dry	0.1	0.5	60	MacDonald et al. 2000
			Congener IUPAC # 170	µg/kg dry	0.07	0.5	60	MacDonald et al. 2000
			Congener IUPAC # 180	µg/kg dry	0.08	0.5	60	MacDonald et al. 2000
			Congener IUPAC # 183	µg/kg dry	0.06	0.5	60	MacDonald et al. 2000
			Congener IUPAC # 187	µg/kg dry	0.09	0.5	60	MacDonald et al. 2000
			Congener IUPAC # 206	µg/kg dry	0.08	0.5	60	MacDonald et al. 2000
	Chlorinated pesticides	EPA 8081B	Aldrin	µg/kg dry	0.2	1	2	Persaud et al. 1993
			alpha-BHC	µg/kg dry	0.1	1	6	Persaud et al. 1993

Table 2-2 Analytical Methods and Performance Criteria for Analysis of Sediment and Biological Tissue Samples for Eighteenmile Creek, Niagara County, New York

Sample Type	Analysis Description	Method	Analyte	Units	Method Detection Limit	Method Reporting Limit	Screening Level	
							Value	Source
			beta-BHC	µg/kg dry	0.2	1	5	Persaud et al. 1993
			gamma-BHC (lindane)	µg/kg dry	0.08	1	2.4	MacDonald et al. 2000
			alpha-chlordane	µg/kg dry	0.1	1	3.2	MacDonald et al. 2000
			gamma-chlordane	µg/kg dry	0.09	1	3.2	MacDonald et al. 2000
			chlordane	µg/kg dry	2	10	3.2	MacDonald et al. 2000
			4,4'-DDD	µg/kg dry	0.1	1	4.9	MacDonald et al. 2000
			4,4'-DDE	µg/kg dry	0.1	1	3.2	MacDonald et al. 2000
			4,4'-DDT	µg/kg dry	0.2	1	4.2	MacDonald et al. 2000
			Dieldrin	µg/kg dry	0.1	1	1.9	MacDonald et al. 2000
			Endosulfan I	µg/kg dry	0.06	1	0.6	NYSDEC 1999 for endosulfan, 2% OC
			Endosulfan II	µg/kg dry	0.1	1	0.6	NYSDEC 1999 for endosulfan, 2% OC
			Endosulfan sulfate	µg/kg dry	0.1	1	0.6	NYSDEC 1999 for endosulfan, 2% OC
			Endrin	µg/kg dry	0.09	1	2.2	MacDonald et al. 2000
			Endrin aldehyde	µg/kg dry	0.1	1	2.2	MacDonald et al. 2000
			Endrin ketone	µg/kg dry	0.09	1	2.2	MacDonald et al. 2000
			Heptachlor	µg/kg dry	0.1	1	2.5	MacDonald et al. 2000
			Heptachlor epoxide	µg/kg dry	0.08	1	5	Persaud et al. 1993
			Hexachlorobenzene	µg/kg dry	0.2	1	20	Persaud et al. 1993
			Methoxychlor	µg/kg dry	0.2	1	12	NYSDEC 1999 for 2% OC
			Toxaphene	µg/kg dry	5	50	0.2	NYSDEC 1999 for 2% OC
	Density, wet	ASTM D854	na	na	na	na	na	na
	Total metals (TAL list)	EPA 6010B (ICP-AES)	Aluminum	mg/kg dry	6	10	58000	MacDonald et al. 1999
		EPA 200.8 (ICP-MS)	Antimony	mg/kg dry	0.02	0.05	2.9	MacDonald et al. 1999; PAETA
		EPA 200.8 (ICP-MS)	Arsenic	mg/kg dry	0.06	0.5	9.8	MacDonald et al. 2000

Table 2-2 Analytical Methods and Performance Criteria for Analysis of Sediment and Biological Tissue Samples for Eighteenmile Creek, Niagara County, New York

Sample Type	Analysis Description	Method	Analyte	Units	Method Detection Limit	Method Reporting Limit	Screening Level	
							Value	Source
		EPA 200.8 (ICP-MS)	Barium	mg/kg dry	0.005	0.05	na	na
		EPA 200.8 (ICP-MS)	Beryllium	mg/kg dry	0.003	0.02	na	na
		EPA 200.8 (ICP-MS)	Cadmium	mg/kg dry	0.004	0.02	1	MacDonald et al. 2000
		EPA 6010B (ICP-AES)	Calcium	mg/kg dry	2	10	na	na
		EPA 200.8 (ICP-MS)	Chromium	mg/kg dry	0.03	0.2	43.4	MacDonald et al. 2000
		EPA 200.8 (ICP-MS)	Cobalt	mg/kg dry	0.003	0.02	50	MacDonald et al. 1999; criterion, Ont.
		EPA 200.8 (ICP-MS)	Copper	mg/kg dry	0.08	0.1	31.6	MacDonald et al. 2000
		EPA 6010B (ICP-AES)	Iron	mg/kg dry	0.3	4	2000	Persaud et al. 1993
		EPA 200.8 (ICP-MS)	Lead	mg/kg dry	0.009	0.05	35.8	MacDonald et al. 2000
		EPA 6010B (ICP-AES)	Magnesium	mg/kg dry	0.04	4	na	na
		EPA 200.8 (ICP-MS)	Manganese	mg/kg dry	0.03	0.1	460	Persaud et al. 1993
		EPA 7471B	Mercury	mg/kg dry	0.002	0.02	0.18	MacDonald et al. 2000
		EPA 200.8 (ICP-MS)	Nickel	mg/kg dry	0.03	0.2	22.7	MacDonald et al. 2000
		EPA 6010B (ICP-AES)	Potassium	mg/kg dry	5	80	na	na
		EPA 200.8 (ICP-MS)	Selenium	mg/kg dry	0.2	1	5	MacDonald et al. 1999; criterion, B.C.
		EPA 200.8 (ICP-MS)	Silver	mg/kg dry	0.008	0.02	0.5	USEPA 2003; ESL

Table 2-2 Analytical Methods and Performance Criteria for Analysis of Sediment and Biological Tissue Samples for Eighteenmile Creek, Niagara County, New York

Sample Type	Analysis Description	Method	Analyte	Units	Method Detection Limit	Method Reporting Limit	Screening Level	
							Value	Source
		EPA 6010B (ICP-AES)	Sodium	mg/kg dry	4	40	na	na
		EPA 200.8 (ICP-MS)	Thallium	mg/kg dry	0.003	0.02	na	na
		EPA 200.8 (ICP-MS)	Vanadium	mg/kg dry	0.02	0.2	na	na
		EPA 200.8 (ICP-MS)	Zinc	mg/kg dry	0.2	0.5	121	MacDonald et al. 2000
	AVS/SEM	EPA (1991) draft method for determining AVS/SEM in sediment and EPA 6010, 6020, and 7471 for metals analysis.	Sulfide (AVS)	µmol/g dry	0.09	0.5	0.01	Target Reporting Level (RL)
			Cadmium (SEM)	µmol/g dry	0.0002	0.0004	0.01	Target RL
			Copper (SEM	µmol/g dry	0.0008	0.002	0.01	Target RL
			Lead (SEM)	µmol/g dry	0.0008	0.002	0.01	Target RL
			Mercury (SEM)	µmol/g dry	0.000005	0.00001	0.01	Target RL
			Nickel (SEM)	µmol/g dry	0.0004	0.003	0.01	Target RL
			Zinc (SEM)	µmol/g dry	0.0009	0.002	0.01	Target RL
Sediment Toxicity	Toxicity, *Hyalella azteca* (10-day)	EPA 100.1	Survival	%	na	na	20%	Maximum allowable control mortality.
			Growth	mg	na	na	Measurable	See Table 11.3 in USEPA (2000).
	Toxicity, *Chironomus dilutus* (10-day)	EPA 100.2	Survival	%	na	na	30%	Maximum allowable control mortality.
			Growth	mg	na	na	0.48 AFDW	Minimum average size at end of test.

Table 2-2 Analytical Methods and Performance Criteria for Analysis of Sediment and Biological Tissue Samples for Eighteenmile Creek, Niagara County, New York

Sample Type	Analysis Description	Method	Analyte	Units	Method Detection Limit	Method Reporting Limit	Screening Level	
							Value	Source
PAC Bioaccumulation Reduction Pilot Study								
Bioaccumulation Testing[c]	4-day screening test with *Lumbriculus variegatus*	EPA 100.3	Survival	%	na	na	Test acceptability requirements in USEPA (2000, Table 13.4)	
	Prepare PAC treatments for 28-day bioaccumulation tests	na	na	na	na	na	na	na
	28-day bioaccumulation test with *Lumbriculus variegatus*	EPA 100.3	Bioaccumulation	µg/kg wet	na	na	Test acceptability requirements in USEPA (2000, Table 13.4)	
Lumbriculus tissue	Lipids	See note d.	Lipid	% wet wt.	na	0.1	0.2	na
	PCB Congeners and Aroclors (8082 list)	EPA 8082	Aroclor 1016	µg/kg wet	2.4	10	25	Target RL based on past studies
			Aroclor 1221	µg/kg wet	2.4	20	25	Target RL based on past studies
			Aroclor 1232	µg/kg wet	2.4	10	25	Target RL based on past studies
			Aroclor 1242	µg/kg wet	2.4	10	25	Target RL based on past studies
			Aroclor 1248	µg/kg wet	2.4	10	25	Target RL based on past studies
			Aroclor 1254	µg/kg wet	2.4	10	25	Target RL based on past studies
			Aroclor 1260	µg/kg wet	2.4	10	25	Target RL based on past studies
			Congener IUPAC # 1	µg/kg wet	1.5	5	0.5	Target RL based on past studies
			Congener IUPAC # 5	µg/kg wet	0.1	0.5	0.5	Target RL based on past studies
			Congener IUPAC # 18	µg/kg wet	0.1	0.5	0.5	Target RL based on past studies
			Congener IUPAC # 31	µg/kg wet	0.1	0.5	0.5	Target RL based on past studies

Table 2-2 Analytical Methods and Performance Criteria for Analysis of Sediment and Biological Tissue Samples for Eighteenmile Creek, Niagara County, New York

Sample Type	Analysis Description	Method	Analyte	Units	Method Detection Limit	Method Reporting Limit	Screening Level	
							Value	Source
			Congener IUPAC # 44	µg/kg wet	0.3	0.5	0.5	Target RL based on past studies
			Congener IUPAC # 52	µg/kg wet	0.4	0.5	0.5	Target RL based on past studies
			Congener IUPAC # 66	µg/kg wet	0.6	1	0.5	Target RL based on past studies
			Congener IUPAC # 87	µg/kg wet	0.2	0.5	0.5	Target RL based on past studies
			Congener IUPAC # 101	µg/kg wet	0.4	0.5	0.5	Target RL based on past studies
			Congener IUPAC # 110	µg/kg wet	0.3	0.5	0.5	Target RL based on past studies
			Congener IUPAC # 138	µg/kg wet	0.09	0.5	0.5	Target RL based on past studies
			Congener IUPAC # 141	µg/kg wet	0.5	0.5	0.5	Target RL based on past studies
			Congener IUPAC # 151	µg/kg wet	0.09	0.5	0.5	Target RL based on past studies
			Congener IUPAC # 153	µg/kg wet	0.1	0.5	0.5	Target RL based on past studies
			Congener IUPAC # 170	µg/kg wet	0.4	0.5	0.5	Target RL based on past studies
			Congener IUPAC # 180	µg/kg wet	0.3	0.5	0.5	Target RL based on past studies
			Congener IUPAC # 183	µg/kg wet	0.2	0.5	0.5	Target RL based on past studies
			Congener IUPAC # 187	µg/kg wet	0.08	0.5	0.5	Target RL based on past studies
			Congener IUPAC # 206	µg/kg wet	0.2	0.5	0.5	Target RL based on past studies

Table 2-2 Analytical Methods and Performance Criteria for Analysis of Sediment and Biological Tissue Samples for Eighteenmile Creek, Niagara County, New York

Sample Type	Analysis Description	Method	Analyte	Units	Method Detection Limit	Method Reporting Limit	Screening Level	
							Value	Source
Sediment	Total organic carbon	See note d.	Total Organic Carbon	% dry wt.	0.04	0.1	1	Persaud et al. 1993
	PCB Congeners and Aroclors (8082 list)	EPA 8082	Aroclor 1016	µg/kg dry	2.1	10	60	MacDonald et al. 2000
			Aroclor 1221	µg/kg dry	2.1	20	60	MacDonald et al. 2000
			Aroclor 1232	µg/kg dry	2.1	10	60	MacDonald et al. 2000
			Aroclor 1242	µg/kg dry	2.1	10	60	MacDonald et al. 2000
			Aroclor 1248	µg/kg dry	2.1	10	60	MacDonald et al. 2000
			Aroclor 1254	µg/kg dry	2.1	10	60	MacDonald et al. 2000
			Aroclor 1260	µg/kg dry	2.1	10	60	MacDonald et al. 2000
			Congener IUPAC # 1	µg/kg dry	1.2	5	60	MacDonald et al. 2000
			Congener IUPAC # 5	µg/kg dry	0.2	0.5	60	MacDonald et al. 2000
			Congener IUPAC # 18	µg/kg dry	0.09	0.5	60	MacDonald et al. 2000
			Congener IUPAC # 31	µg/kg dry	0.07	0.5	60	MacDonald et al. 2000
			Congener IUPAC # 44	µg/kg dry	0.3	0.5	60	MacDonald et al. 2000
			Congener IUPAC # 52	µg/kg dry	0.2	0.5	60	MacDonald et al. 2000
			Congener IUPAC # 66	µg/kg dry	0.1	1	60	MacDonald et al. 2000
			Congener IUPAC # 87	µg/kg dry	0.2	0.5	60	MacDonald et al. 2000
			Congener IUPAC # 101	µg/kg dry	0.08	0.5	60	MacDonald et al. 2000
			Congener IUPAC # 110	µg/kg dry	0.08	0.5	60	MacDonald et al. 2000
			Congener IUPAC # 138	µg/kg dry	0.08	0.5	60	MacDonald et al. 2000
			Congener IUPAC # 141	µg/kg dry	0.06	0.5	60	MacDonald et al. 2000
			Congener IUPAC # 151	µg/kg dry	0.07	0.5	60	MacDonald et al. 2000
			Congener IUPAC # 153	µg/kg dry	0.1	0.5	60	MacDonald et al. 2000
			Congener IUPAC # 170	µg/kg dry	0.07	0.5	60	MacDonald et al. 2000
			Congener IUPAC # 180	µg/kg dry	0.08	0.5	60	MacDonald et al. 2000

Table 2-2 Analytical Methods and Performance Criteria for Analysis of Sediment and Biological Tissue Samples for Eighteenmile Creek, Niagara County, New York

Sample Type	Analysis Description	Method	Analyte	Units	Method Detection Limit	Method Reporting Limit	Screening Level	
							Value	Source
			Congener IUPAC # 183	µg/kg dry	0.06	0.5	60	MacDonald et al. 2000
			Congener IUPAC # 187	µg/kg dry	0.09	0.5	60	MacDonald et al. 2000
			Congener IUPAC # 206	µg/kg dry	0.08	0.5	60	MacDonald et al. 2000
	Percent solids	ASTM D2216	% solids	%	na	0.1	1	na

Notes:
[a] 20 gamefish (15 bullhead + 5 largemouth bass) skin-on fillet samples, 15 forage fish composite samples, and nine crayfish composite samples.
[b] Three original samples and one field duplicate.
[c] Four (4) PAC treatments (5%, 3%, 1%, and 0% [control]) will be prepared from each of the three (3) sediment samples. See scope of work and background information for further detail.
[d] As per laboratory SOP (see Appendix B).

Key:
AFDW = Ash free dry weight
AOC = Area of Concern
AVS = Acid volatile sulfide
B.C. = British Columbia, Canada
IUPAC = International Union of Pure and Applied Chemists
Na = Not applicable
OC = Organic carbon
Ont. = Ontario, Canada
PAC = Powdered activated carbon
PAET = Probable apparent effect threshold approach
PCB = Polychlorinated biphenyl
RL = Reporting level
SEM = Simultaneously extracted metals
TAL = Target analyte list
USEPA = United States Environmental Protection Agency

Table 2-3 Field Quality Control Samples for Project

QC Sample	Description	Acceptance Criteria	Corrective Action
Field Duplicate	One per 20 sediment samples for each analysis. None required for fish and crayfish.	RPDs of 70% for sediment	Qualify related samples and asses data usability

Table 2-4 Laboratory Quality Control Checks for Project

Methods	Quality Check	Frequency	Acceptance Criteria	Corrective Action
All methods	MB	One per matrix per preparation batch.	The goal is for method blanks to be free of contamination. Low-level contamination may be present, but must be less than the reporting limit.	If contamination is greater, samples are reanalyzed. If contaminants are present in the method blank but not in project samples, no further action is required.
All methods	LCS	One per matrix per preparation batch for each analysis. The LCS must contain all target analytes of concern at the site.	The LCS recovery must be within method control limits to demonstrate acceptable method performance. Sporadic marginal failures of a few target analytes reported when greater than five target analytes are allowed.	If LCS recoveries are outside QC criteria for more than a few target analytes, recoveries are significantly low, or the compounds were detected in the samples, then corrective action is required. Correcting actions are identified in Section 3.1.4

Table 2-4 Laboratory Quality Control Checks for Project

Methods	Quality Check	Frequency	Acceptance Criteria	Corrective Action
Organic Methods	Surrogate Spikes	All samples and QC checks.	Surrogate recoveries must be within QC criteria for method blanks and LCSs to demonstrate acceptable method performance. Surrogate recoveries that are outside QC criteria for a sample indicate a potential matrix effect.	If surrogate recoveries are outside QC criteria for method blanks or LCSs, corrective action is required and the Project Chemist should be notified. Matrix effects must be verified based on review of recoveries in the method blank or LCS, sample reanalysis, or evaluation of interfering compounds.
All methods	MS/MSD	One per matrix per SDG for each analysis. The spike solution must contain a broad range of the analytes of concern at the site. The overall frequency of MS/MSD on project samples must be at least one set per 20 samples.	Method QC criteria or LCS criteria if not specified. MS recoveries outside the control limits applied to the LCS indicate matrix effects. QC criteria for MSD RPDs are 20% for waters and 35% for soils and sediments unless the laboratory provides additional statistical criteria.	Sample clean-up procedures may be warranted for samples with severe matrix effects.

Key:

LCS = Laboratory Control Sample
MB = Method Blank
MS/MD = Matrix Spike/Matrix Duplicate
SDG = Sample Delivery Group

Table 2-5 Field Equipment and Calibration Procedures

Instrument or Equipment	Description[a]	Field Calibration Procedure	Acceptability/ Performance Criteria	Responsible Personnel
Water Quality Meter for pH/Conductivity, Temperature, DO, Oxidation Reduction (REDOX)	Meter designed for field use with battery operation. The unit must contain separate pH, temperature, conductivity, DO, and ORP probes in one unit.	Before use, pH, specific conductance, DO, and ORP probes need to be calibrated or tested for responsiveness. The pH probe will be calibrated first. This is done by placing the probe in pH 7, then pH 4, standard solutions and adjusting the pH calibration knobs until the correct measurement is obtained. The ORP probe is then calibrated with the ORP standard solution (Zobell), and the DO probe is checked in accordance with manufacturer guidelines. The probes should be rinsed with deionized water between each calibration solution and following calibration. Used calibration solution is to be discarded. Finally, the conductivity probe is checked with a solution of known conductivity.	Turbidity and DO ± 10% pH ± 0.01 pH Conductivity at ± 2% FSD The instrument will be checked with a pH standard every 4 hours and at the end of the sampling day. If the response is greater than 0.2 units more or less than the standard, complete calibration will be conducted.	Project Biologist, Sampler
Turbidity Meter	Nephelometer designed for field use with battery operation. Range 0.01 to 1,000 NTU.	The unit is factory calibrated. Field procedures involve checking the unit's responsiveness at least once a day using factory supplied standards. The responsiveness should be checked on the 0 to 10 range, 0 to 100 range, and 0 to 1,000 range.	± 10%	Project Biologist, Sampler

[a] Description is for typical equipment; equivalent units may be used.

Key:
DO = dissolved oxygen
NTU = nephelometric turbidity unit
ORP = oxygen reduction potential

2.8 Inspection/Acceptance of Supplies and Consumables

Procedures for the procurement, inspection, maintenance, and management of equipment and supplies for project activities are documented in E & E's Government Property Procurement SOP. All field supplies and equipment will be procured as part of the contract and maintained by the technical team. Supplies and equipment will be inspected on receipt at the site to verify that the correct materials were received. Field personnel will be responsible for performing inspections. The following is a list of critical supplies for the project:

- Electrofishing equipment (wands, voltage regulator, and generator);
- Sample containers;
- Jon boat with outboard motor; and
- Ekman and Ponar dredges.

Consumables (e.g., coolers, ice, aluminum foil, plastic Ziploc bags, and permanent markers) that will be used for this investigation are readily available at minimal cost from a number of sources and are not considered critical.

2.9 Non-direct Measurements

For data acquired from non-direct measurement, sources include the following (see Table 2-6):

- Physical information, such as descriptions of sampling activities and sediment characteristics;

- State, federal, and local environmental agency reports;

- Reference computer databases and literature files; and

- Historic reports on site and subjective information gathered through discussions.

Data from non-direct measurements will be reviewed and summarized in the final report. Data from all non-direct measurement sources are stored as indicated in Section 1.6. Additional data from non-direct measurements will be reviewed and used as indicated in Section 1.3 and Appendix A.

Table 2-6 Summary of Data from Non-Direct Measurements

Data Source	Description[a]	Intended Use	Acceptability/ Performance Criteria
New York State GIS Service	GIS Layers for Niagara County and aerial imagery	Present parcel data, permit locations, hazardous waste site locations, and aerial images for evaluation of future site sampling	New York State site will be checked prior to data use to verify most current copy.
Historic Data	Historic investigations currently loaded into GIS and Project Database	Data assessment	Data were checked and compiled under previous tasks for USEPA and USACE.
NYSDEC regulations and literature sources	Site screening values for sediment, surface water, and soil concentrations.	Screening values will be used as part of the planning process to establish appropriate DQOs.	Values will be reviewed against current source to verify the values are still acceptable.

Note:
[a] Description is for typical data type and not specific to each data layer.

Key:
DQO = data quality objective
GIS = geographic information system
NYSDEC = New York State Department of Environmental Conservation
USACE = United States Army Corps of Engineers
USEPA = United States Environmental Protection Agency

2.10 Data Management

Data management procedures track samples and results from work plan generation to the final report. The field data include approved work planning tables, labels, field sampling forms, COC forms, and logbooks. The surveyor will provide coordinates for all sample locations. The field team leader will review all field data for accuracy. Any field data not provided by the laboratory will be entered into a database or spreadsheet.

Electronic data will be provided in accordance with the most recent version of NYSDEC standardized EQuis EDD format.

The E & E technical team will process the EDD to verify that criteria established in this QAPP (Tables 2-2 to 2-4 and Appendix B) are met. The Project Chemist will review all laboratory and field data to verify the results against the hard copy and check for transcription errors. The Project Chemist will verify qualifiers added by data processing and add any data qualifiers. The individual Sample Delivery Group (SDG) EDD files will be processed to a centralized data management system to store all reviewed and approved data. Data that will appear on data tables for the report will be generated from the centralized database, which will serve as the central, protected data source for all data handling operations.

The central database will be stored in a secure area on E & E's network with access limited to data management specialists designated by the Project Manager. The central database can be

electronically linked to E & E's geographic information system (GIS) systems, risk assessment programs, and other final data user models and statistical programs. Data users may enter additional electronic data, such as risk-based criteria, for comparison of results. This data will be stored in separate tables in the database and linked to the actual results. Any data from outside sources will include a description of the data, a reference to the source, and the date updated. Outside data will be checked prior to use to verify that current values are used. The central database will be used to create tables for the final report.

3 Assessment and Oversight

3.1 Assessment and Response Actions

Technical assessment activities include peer review, data quality reviews, and technical system audits. Both overall and direct technical assessment activities may result in the need for corrective action. The project approach for implementing a corrective action response program for both field and laboratory situations is summarized briefly in the following subsections. The project QA Officer has stop-work authority on any project activities that may have negative quality impacts prior to completion of corrective actions.

3.1.1 Peer Review

Peer review for all project deliverables, such as the QAPP, draft and final reports, and technical memoranda will be implemented. The peer review process provides a critical evaluation of the deliverable by an individual or team to determine if the deliverable will meet established criteria, quality objectives, technical standards, and contractual obligations. The Project Manager will assign peer reviewers when the publications schedule is established. The task leads will be responsible for ensuring all peer reviewers participate in the review process and approve all final deliverables. Peer reviewers are knowledgeable of the material, but remain independent of the technical processes that generated the document.

All technical reports submitted to the client will be released through E & E's publications department. The publications department is responsible for overseeing the document production process, including the preparation, review, approval, release, and distribution of documents and the incorporation of any changes. Documents will be prepared with document control blocks identifying the section of the report, the revision number, and the date. Each time a change is made, the document will be reviewed and approved as described below.

- Technical staff will prepare reports and provide to the publications department for processing;
- Publications will assign an editor to the report. The editor will provide a review copy with track changes locked to the peer reviewer;
- Peer reviewers will provide comments in electronic format to the Project Manager;

- The Project Manager will address the comments, return the report to the technical staff for update to address the comments, or note that the comment cannot be addressed based on the project scope; and

- The editor will receive a final reviewed copy for processing and will verify that all comments have been addressed.

In addition to the internal peer review, external peer review will be performed during review of the draft deliverables. The Project Manager will track comments from external reviewers and develop a response to comments to include with the final report to document how all external comments were addressed in the final report. If the response to external comments results in significant changes to the report, the Project Manager will provide a formal response to comments to the client for review and approval prior to issuing the final report.

3.1.2 Data Quality Assessments

Data quality assessments performed on data generated by the entire project team are described in Section 4. The quality of data from secondary sources that are inputs to the data evaluation process will be assessed by the E & E technical team as described in the SOP for review of data from secondary sources. In general sources that have been verified in previous peer reviewed studies will be considered acceptable. New sources that are identified as part stakeholder input will be assessed to verify data are generated within parameters of the scientific process applicable to that field of study, such as peer reviewed academic studies or projects overseen by knowledgeable state or federal agencies.

3.1.3 Technical Systems Assessments

The entire project team is responsible for the ongoing assessment of the technical work performed by the team, identification of nonconformance with the project objectives, and initiation, implementation, and documentation of corrective action. Independent performance reviews are technical assessments that are a possible part of the QA/QC program. The types of assessments, frequency, and personnel responsible are summarized in Table 3-1.

Table 3-1 Project Assessments and Reports

Assessment or Report Type	Frequency	Party(ies) Performing Assessment	Party(ies) Receiving Assessment Findings	Party(ies) Responsible for Corrective Actions
QAPP Review	Project startup	Project Managers, QA Officers, field lead and laboratories	See Distribution List	E & E QA Officer
Field Inspection	Once in field program	E & E Project Manager	Included in draft report	E & E Field Task Lead
Deliverable Review	Prior to each report release	Peer Reviewers	E & E Project Manager	E & E Project Manager or Task Leads

3.1.4 Corrective Action

Corrective actions will be implemented as needed based on data quality and technical assessments. The Project Manager is responsible for initiating corrective action and obtaining approval from the client and QA Officer. After a corrective action has been implemented, its effectiveness will be verified. If the action does not resolve the problem, appropriate personnel will be assigned to investigate and effectively remediate the problem. Specific corrective actions will be clearly documented in the logbooks, monthly progress reports or technical reports.

The corrective action process for field or laboratory situations are described below.

Field Situations

The need for corrective action in the field may be determined by technical assessments or by more direct means, such as equipment malfunction. Immediate corrective actions taken in the field will be documented in the project logbook. Corrective actions may include, but are not limited to:

- Recalibrating field instruments and checking battery charge;
- Training field laboratory personnel in correct procedures; and
- Accepting data with an acknowledged level of uncertainty.

Laboratory Situations

Out-of-control QC data, laboratory audits, or outside data review may determine the need for corrective action in the laboratory. Corrective actions implemented as part of the standard laboratory practice will be documented in the case narrative. Corrective actions directed by E & E based on data assessment may include, but are not limited to:

- Reanalyzing samples, if holding times permit; or
- Accepting data with an acknowledged level of uncertainty.

The laboratory corrective actions are defined in analytical SOPs included in Appendix C. Any deviations from approved procedures must be documented and approved by the Project Chemist.

3.2 Reports to Management

Reports that will be given to management include, as appropriate:

- **Project Status Reports.** Project status reports are completed by the Project Manager to document the overall assessment of the project on a monthly basis.

A comprehensive technical report that summarizes work activities and provides a data evaluation will be submitted. A discussion of the validity of results in the context of QA/QC procedures will be made and the data usability summary report (DUSR) will be provided.

4 Data Validation and Usability

4.1 Data Review, Validation, and Verification Requirements

All data will be reviewed against the QA/QC requirements in the QAPP. Non-conformance issues or deficiencies that could affect the precision and accuracy of the reported results are identified and considered when assessing whether the result is sufficient to achieve DQOs. Other data collected as part of this project will be evaluated for consistency with this QAPP. Data review checks for the project are described below:

- Field data, such as sample identifications and sample dates, will be checked against the planned field activities and the laboratory report.

- QC checks of field GPS data will be performed by the GIS personnel. GIS personnel will check that survey standards established in the planning phase were correctly followed, including verifying that horizontal and vertical controls were properly established. GPS data will be sorted and reduced to an X, Y file and the sorted data will be point-plotted, contoured, and annotated on geo-referenced GIS drawings.

- Biological and ecological data will be reviewed by internal experts for consistency with literature values and known data for the site. Any differences in results will be evaluated based on upon sample dynamics and/or the site conditions. If the results are substantially different, additional data collection may be recommended to resolve inconsistencies.

- Analytical reporting limits and target compounds and QC summary data for surrogates, method blanks, laboratory control samples (LCS), and matrix spike/matrix spike duplicate (MS/MSD) samples will be compared to limits listed tables in Sections 1.4, 2.4, and 2.5 by the Project Chemist as described in Section 4.2.

- Calibration summary data will be checked by the laboratory to verify that all positive results for target compounds were generated under an acceptable calibration as defined by the analytical method. Any deviations will be noted in the case narrative and reviewed by the Project Chemist.

- Modeling or data calculations will be checked by an independent technical reviewer. The reviewer will verify the correct data inputs, perform representative re-calculations if applicable, and verify the outputs are consistent with known or expected values. If the

results are substantially different, additional data evaluation may be recommended to resolve inconsistencies.

4.2 Validation and Verification Methods

The laboratory is responsible for performing internal data review. All levels of laboratory review must be fully documented and available for review if requested. After receipt from the laboratory, project data will be validated using the following steps:

Evaluation of Completeness

The Project Chemist checks the electronic files for compliance with standard format and the QAPP. If errors in loading are found, the EDD files will be returned to the laboratory. The Project Chemist also verifies that the laboratory information matches the field information and that the following items are included in the hard copy data package:

- COC forms and Sample Summary forms;
- Case narrative describing any out-of-control events and summarizing analytical procedures;
- Data report forms (i.e., Form I);
- QA/QC summary forms; and
- Chromatograms documenting any QC problems.

If the data package is incomplete, the Project Chemist will contact the laboratory, which must provide all missing information within one day.

Evaluation of Compliance

The validation procedures process the electronic data and assign qualifiers if outliers are found. As relevant, the project chemist will follow SOPs for data review and complete checklists. Additional compliance checks on representative portions of the data are briefly outlined below:

- Review chromatograms and other raw data if provided as backup information for any apparent QC anomalies;
- Ensure that all analytical problems and corrections are reported in the case narrative and that appropriate laboratory qualifiers are added;
- For any problems identified, review concerns with the laboratory, obtain additional information if necessary, and check all related data to determine the extent of the error; and
- Non-analytical data on field data sheets will be reviewed as outlined in Section 2.2.

Project chemists will follow qualification guidelines in EPA Region 2 data validation SOPs or EPA Contract Laboratory Procedure (CLP) National Functional Guidelines for Organic Data Review, EPA 540/R-99-008 (October 1999) or EPA CLP National Functional Guidelines for Inorganic Data Review, EPA 540-R-04-004 (October 2004), but will use the specific method criteria for evaluation. The DUSR will be completed as specified in NYSDEC Guidance of the Development of DUSRs (July 1999). E & E data validation criteria are incorporated into checklists for some methods.

Data Review Reporting

The Project Chemist or reviewer of non-analytical data will perform the following reporting functions:

- Alert the Project Manager to any QC problems, obvious anomalous values, or discrepancies between the field and laboratory data, that may impact data usability;
- Discuss QC problems in a DUSR for each laboratory report;
- Prepare analytical data summary tables of qualified data that summarize those samples and analytes for which detectable concentrations were exhibited including field QC samples; and
- At the completion of all field and laboratory efforts, summarize planned versus actual field and laboratory activities and data usability concerns in the technical report.

4.3 Reconciliation with User Requirements

Any deviations from analytical performance criteria or quality objectives for the project will be documented in the DUSR provided to the data users for the project.

The QA Officer or Project Chemist will work with the final users of the data in performing data quality assessments. The data quality assessment may include some or all of the following steps:

- Data that are determined to be incomplete or not usable for the project will be discussed with the project team. If critical data points are involved which impact the ability to complete project objectives, data users will report immediately to the Project Manager. The Project Manager will discuss resolution of the issue with client and implement necessary corrective actions (for example re-sampling);
- Data that are non-detect ,but have elevated reporting limits due to blank contamination or matrix interference, will be compared to background values. If reporting limits exceed the background values, then results will be handled as incomplete data as described above; and
- Data that are qualified as estimated will be used for all project decision making. Data assessors comparing to results to background will have to account for the higher level of uncertainty in their statistical analysis.

Part of the data assessment process involves comparing results to known or expected values; regulatory criteria or standards, and/or background concentrations. The following data assessment procedures are anticipated for this project.

- Comparing fish and crayfish chemical residues among reaches in Eighteenmile Creek, comparing chemical residues among species within reaches, comparing fish and crayfish chemical residues to appropriate screening levels (see Tables 1-2 and 2-2), and comparing fish chemical residues to those from earlier investigations;

- Comparing sediment contaminant data to appropriate screening levels (see Table 2-2) and results from earlier investigations; and

- Comparing bioaccumulation test results among reaches and with results from previous investigations.

In addition, data collected under this QAPP can be used to evaluate the status of BUIs 3, 5, and 6 (see Table 1-2). The PCB Aroclor and congener fish residue data will be used to estimate total PCB residue concentrations for fish samples from Eighteenmile Creek. Two totals will be calculated for each sample, one based on the sum of Aroclors and one based on the sum of congeners. Total PCB levels in each fish species will be compared with the PCB fish tissue criterion using a one-sample t-test. This version of the t-test can be used to compare a single observation (e.g., PCB fish-tissue criterion) with a group (e.g., five brown bullhead samples from Reach 1 in Eighteenmile Creek) to determine if the single observation and group are, or are not, from the same population. BUIs 3 and 5 include delisting criteria that involve comparing fish-tissue PCB concentrations with fish-tissue criteria (see Table 1-2).

The first and second delisting criteria for BUI 6 (Degradation of Benthos) indicate how benthic macroinvertebrate community data should be used to evaluate this BUI (see Table 1-2). The third delisting criterion for BUI 6 indicates how sediment toxicity testing data should be used to evaluate this BUI (see Table 1-2). The sediment chemistry data collected as part of the current investigation will be used in conjunction with the benthic-community and sediment-toxicity data to identify possible causes of impairment. For example, a chemical that is correlated with toxicity and also exceeds its respective sediment screening level (see Table 2-2) may be contributing to the observed toxicity.

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A Field SOPs

ecology and environment, inc.

STANDARD OPERATING PROCEDURE

FIELD ACTIVITY LOGBOOKS

SOP NUMBER: DOC 2.1

REVISION DATE: 5/25/2012 **SCHEDULED REVIEW DATE: 5/25/2017**

Contents

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1 Scope and Application

Proper documentation of field activities is a critical component of any field effort. This Standard Operating Procedure (SOP) establishes procedures for initiating, entering information/data into, reviewing, and maintaining/storing hard copy field logbooks for E & E field activities. Field activities may range from simple reconnaissance to complex sampling programs and may include: visual or other observations, in situ or ex situ field measurements (monitoring), or sample collection, and can include meetings with E & E clients, sub-contractors or other stakeholders.

Field logbook documentation may be supplemented by other records (e.g., site safety forms, data collection forms, electronic data, or geotechnical logbooks). Information and data to be recorded on such forms or logbooks are addressed in the applicable SOPs.

Field observations, measurements, and samples have value to data users only to the degree that the observation, measurement, or sample is representative of a specified environment, setting, or process. Field logbooks address representativeness by documenting the following:

- Identification of the subject of the observation, measurement, or sampling;
- Selection of an observation, measurement, or sampling location and time that represents that subject;
- Compliance with or deviation from the work plan, sampling and analysis plan, quality assurance project plan, or other project or program plans; and

Sufficient documentation of how the observation, measurement, or sample represents the same subject as other observations, measurements, or samples from the vicinity. Complete and accurate logbook entries are important for several reasons: to ensure that data collection associated with field activities is sufficient to support the successful completion of the project; to provide sufficient information so that someone not associated with the project can independently reconstruct the field activities at a later date; to maintain quality control throughout the project; to document changes to or deviations from the work plan; to fulfill administrative needs of the project; and to support potential legal proceedings associated with a specific project. This Field Activity Logbook SOP is intended for use by personnel who have knowledge, training and experience in the field activities being conducted.

2 Definitions and Acronyms

Field	Locations (sites) outside the controlled environment of an office or laboratory.
Field Observation	The qualitative and/or quantitative remarks/statements regarding sensory inputs noted in the field.
Field Measurement	The quantitative determination of physical, chemical, biological, geological or radiological properties of a matrix by measurements made in the field.
Field Sampling	The process of obtaining a representative portion of an environmental matrix suitable for laboratory or field measurement or analysis.
E & E	Ecology and Environment, Inc.
EPA	Environmental Protection Agency

ID	Identification
IDW –	Investigation-derived waste
QA	Quality assurance
QC	Quality control
SOP	Standard operating procedure

3 Procedure Summary

Prior to field activity, the program/project manager identifies field personnel; designates a field team leader; and team members responsible for documenting field activities. Since there may be multiple activities with unique logbooks, there may be multiple team members responsible for documenting field activities.

Prior to entering the field, the individual responsible for documenting field activities or other designated author should briefly summarize the field activities that will be conducted in the logbook.

Visual or other observations, in situ or ex situ field measurements (including instrument/equipment calibrations), or sample collection information should be recorded in real-time as field work is conducted. Meetings, including electronic communications, with E & E, clients, sub-contractors or regulatory personnel should be recorded. Compliance with or deviation from the work plan, sampling and analysis plan, quality assurance project plan, or other project or program plans should be highlighted together with authorization for such deviations.

The field team leader should review log book entries on a daily basis or more frequently, if appropriate. The project/program manager should review the logbooks at the close of fieldwork or more frequently for long-term field events. Logbooks may be audited by quality assurance personnel from E & E or a client.

The program/project manager is responsible for storing/archiving applicable logbooks in the project file.

4 Cautions

Logbook entry must be a priority and not left to “later.” Contemporaneous documentation is critical to accurate and precise reporting.

Field logbooks become part of the permanent record for projects/programs and, thus, should include factual material, not opinions. Language used in logbooks should be objective and factual. Pertinent personal observations may be included, but must be clearly identified as such.

If multiple logbooks are used, a project logbook should be used to maintain control of all other logbooks.

Do not leave blank line(s) between logbook entries. Cross out blank spaces with a single line, initial and date the cross out.

5 Equipment and Supplies

Logbooks must be bound with consecutively numbered pages.

Entries should be made using indelible ink (preferably black).

6 Procedure

The following guidelines are used for completing Field Activity Logbooks:

- Logbooks will be assigned by the program/project manager to the field team leader. Additional logbooks may be assigned to other personnel (e.g., health and safety monitors). The program/project manager is responsible for tracking field event logbooks.
- A separate field logbook must be maintained for each project.
- Logbooks must be bound and contain consecutively numbered pages.
- The first entry for each day will be made on a new, previously blank page.
- No pages may be removed for any reason, even if mutilated or illegible. If a page or portion of a page is accidentally skipped during fieldwork, it should be crossed out, signed, and dated.
- Entries should be made in chronological order. Observations that cannot be recorded during field activities should be recorded as soon as possible. If logbook entries are made after field activities, the time of the activity/observation and the time that it is recorded should be noted.
- The time of each entry should be noted. It is customary to record time using a 24-hour clock.
- If corrections are necessary, they must be made by drawing a single line through the original entry in such a manner that it can still be read. Do not erase or render an incorrect notation illegible. The corrected entry should be written beside the incorrect entry, and the correction initialed and dated. Corrected errors may require a footnote explaining the correction.
- Logbooks should be signed at the end of each day (if more than one person makes entries into the logbook, each person should sign and date next to his or her entries). Signatures should be written along a single diagonal line drawn across the blank portion of the page following the last entry of the day.
- At the completion of the field activity, the logbook must be returned to the project manager to include with the project files.

6.1 Format

The following guidelines provide a general format and required information for all routine field activities using the Field Activity Logbooks:

- Title Page
    - The logbook title page should contain the following items:
    - Site name;
    - Site identification (ID) number; if applicable;
    - Location;
    - Project Number;

  - Start/finish date; and
  - Book of . (may be completed at the end of the project)
- First Page
  - The following items should appear on the first page of the logbook prior to daily field activity entries:
  - Project Number;
  - Date;
  - Summary of proposed work (reference work plan and contract documents, as appropriate);
  - Weather conditions;
  - Team members and duties;
  - Time work began and time of arrival (using 24-hour clock notation); and
  - Arrival/departure times of each field team member and other personnel if different from overall work times.
- Successive Pages
  - In addition to specific activity entries and observations, the following items should appear on every logbook page:
  - Date at the top of each page,
  - Project Number and site name,
  - Weather conditions if changed from the first entry of the data,
  - Signature and date at the bottom of each page (if more than one person makes entries into the logbook, each person should sign and date next to his or her entries); and
  - Strikethroughs of any unused lines. If more than one person makes entries into the logbook, each person should sign and date next to his or her entry.
- Last Page
  - The last page of the logbook may contain a brief paragraph that summarizes the work that was completed in the field and recorded in this logbook.
  - The last page should indicate if work is continuing in subsequent logbooks or if the project is complete.

6.2 Logbook Information

Field logbook entries will contain a variety of information based on the field activities being conducted (e.g., observing, monitoring, or sampling). The specific type of information recorded in the logbook will depend on the project requirements. In general, information recorded on field forms or electronic data do not need to be recorded in the logbook.

- If not field sampling map is available then a site sketch should be included and updated as necessary identifying the site layout, features and points of interest. A north arrow and rough scale should be included,

- A sketch of individual sampling locations if GPS coordinates are not collected,
- GPS locations, as applicable, for site features,
- Physical description of the site as observed during sample collection,
- Weather conditions, updated as necessary,
- Record of phone calls and/or other contacts with individuals at the site; including names and affiliations,
- Daily brief summary of the site safety meeting if not recorded on separate form,
- Daily brief outline of field activities to be performed that day,
- Pertinent field observations and any unique method to gather observations,
- Documentation of photographs, including:
    - Make and model of the camera(s),
    - Description of the photograph including the date and time,
    - Photograph number,
    - Direction or view angle of the photograph,
    - Name of the photographer(s),
- Brief description of monitoring procedures,
- Model and serial numbers of monitoring equipment,
    - Equipment preparation/calibration procedures, date and time, and results if not recorded on separate form,
    - Field maintenance and/or repairs,
- Sample collection procedures and reference to applicable work plan section or SOP,
- Sample collection activities, including:
    - Pre-sampling activities (e.g., well purging and the number of volumes purged before sample collection),
    - Data associated with pre-sampling activities (e.g., well purging pH, conductivity, temperature data),
    - Equipment decontamination procedure,
- Sample information and observations
    - Sample number, station location ID, programmatic ID , and/or location, including relationship to permanent reference point(s),
    - Name(s) of sampler(s),
    - Sample description, sample depth interval, sample time, sample date, and any field screening results,
    - Sample matrix and number of aliquots if the sample is a composite,
    - Container and preservatives used, recipient laboratory including contact information, and requested analyses, and

  - Any preservative added in the field including preservative type, lot number and expiration date.
- Quality assurance (QA)/quality control(QC) samples,
  - For trip blanks indicate the source of the blanks,
  - For equipment rinsate samples, the equipment from which the rinsate sample is collected should be noted and source of the DI water, and
  - Field duplicates or replicates and a description of how the duplicate was sub-sampled.
- Shipping paper (airbill) numbers, chain-of-custody form numbers.

6.3 Work Plan Changes/Deviation

Compliance with or deviation from the work plan, sampling and analysis plan, quality assurance project plan, or other project or program plans should be highlighted together with authorization for any deviations. Deviations (who, what, where, when, why, and how) from the plans and the circumstances necessitating such changes should be recorded.

6.4 Investigation-Derived Waste

Disposition of non-hazardous versus potentially hazardous IDW should be delineated in the field planning documents. The following information should be included in the logbook:

- Nature and disposition of non-hazardous wastes;
- The type and number of containers of potentially hazardous IDW generated (each “drum” should be numbered and its contents noted);
- Information relevant to characterizing IDW;
- Disposition of IDW (left on site or removed from site); and
- IDW sample information should be recorded the same as other samples.
- The type of paperwork that accompanied the waste/sample shipment (e.g., manifests).

6.5 Data Collection Forms

Certain phases of fieldwork may require the use of separate project-specific data collection forms, such as sample collection, equipment calibration or daily summary forms. Use of such forms and the types of information recorded should be noted in logbook. Information recorded on data entry forms does not need to be repeated in the logbook.

7 Quality Assurance/Quality Control

Compliance with or deviation from work plan, sampling and analysis plan, quality assurance project plan, or other project or program plans should be highlighted together with authorization for any deviations.

Prior to field activity, among other responsibilities, the program/project manager should identify knowledgeable, trained, and experienced field personnel; designate a field team leader; and an individual responsible for documenting field activities. Since there may be multiple activities

with unique logbooks, there may be multiple individuals responsible for documenting field activities.

Prior to entering the field, the individual responsible for documenting field activities or other designated author should briefly summarize the field activities being conducted in the logbook.

The field team leader should review log book entries on a daily basis or more frequently if appropriate. The project/program manager should review the logbooks at the close of fieldwork or more frequently for long-term field events. Logbooks may be audited by quality assurance personnel from E & E or a client.

The project/program manager is responsible for storing/archiving applicable logbooks in the project file.

8 Special Project Requirements

Project or program-specific requirements that modify this procedure should be entered in this section and included with the project planning documents.

9 References

The following list sources of technical information on field logbooks.

United States Environmental Protection Agency (EPA). 1988. *Guidance for Conducting Remedial Investigations and Feasibility Studies Under CERCLA Interim Final*, U.S. EPA, EPA/540/G-89/004, October 1988

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END OF SOP

ecology and environment, inc.

STANDARD OPERATING PROCEDURE

AQUATIC SEDIMENT SAMPLING

SOP NUMBER: ENV 3.8

REVISION DATE: 5/25/2012 **SCHEDULED REVIEW DATE: 5/25/2017**

Contents

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1 Scope and Application

This Standard Operating Procedure (SOP) describes the procedures utilized by E & E samplers for collecting representative sediment samples from beneath aquatic environments. The purpose of sediment sampling may range from simple reconnaissance to complex sampling programs. This SOP can be followed for all routine sample collection activities which may include: visual or other observations, in situ or ex situ field measurements (monitoring), or sample collection for biological, chemical, geological, radiological or physical analysis. Site-specific sampling procedures vary depending on the data quality objectives (DQOs) identified in program/project planning documents.

E & E routinely utilizes three types of sediment collection procedures, grab sampling, hand sampling, and coring. Sediment coring can be done by hand or by a contracted driller or vibracore company. For the purposes of this SOP, sediments are those mineral and organic materials situated beneath an aqueous layer. The water may be static, as in lakes, ponds, and impoundments; or flowing, as in rivers and streams.

Procedures for collecting soil samples for volatile organic compound (VOC) analyses are presented in the E & E VOC Soil and Sediment Sampling SOP ENV 25.

Procedures for sample handling are defined in E & E Environmental Sample Handling, Packaging and Shipping SOP ENV 3.16. Site-specific sample handling procedures are dependent on the project DQOs.

Procedures for equipment decontamination are defined in E & E Sampling Equipment Decontamination SOP ENV 3.15. Site-specific equipment decontamination procedures are dependent on the project DQOs.

This aquatic sediment sampling SOP is intended for use by personnel who have knowledge, training and experience in the field sediment sampling activities being conducted.

2 Definitions and Acronyms

cm	centimeters
DQO	data quality objectives
E & E	Ecology and Environment, Inc.
SOP	Standard Operating Procedure

3 Procedure Summary

Hand sampling is generally utilized to collect shallow sediment samples from along freshwater and marine shorelines or wetlands. Grab sampling, as routinely performed by E & E personnel, is conducted by using small vessels/floating platforms or stationary structures (e.g., bridges) above water to collect samples. Corers may be used to collect surface and shallow subsurface sediment along freshwater or marine shorelines, wetlands, or from floating platforms or stationary structures.

Hand sampling is utilized for aquatic surface sediment collection from wetlands; shallow lakes/ponds, shallow, low velocity streams/rivers; and marine intertidal zones. Pre-cleaned spoons, trowels, or other types of scoops are used to collect shallow (usually less than 10

centimeters [cm] deep) sediment samples. Sediment is collected manually from hand dug excavations. The depth interval of sediment collection is identified in the project planning documents.

A modified Van Veen, Ponar, Ekman or equivalent dredge is used for grab aquatic sediment sampling. Pre-cleaned dredges are used to mechanically collect the grab shallow surface sediment samples. Aliquots of sediment are collected by hand from within the body of the dredge. The depth interval of sediment collection is defined based on the type of dredge selected and is usually about 15 cm. The depth of penetration will depend on the type of sediment. The selection of the appropriate dredge should be identified in the project planning documents. In deeper waters, dredges can be used can be deployed from a boat or floating platform using a wench. This work is typically contracted.

Pre-cleaned hand corers or augers are used for shore-based core sample collection. The core tube/auger is advanced into the sediment to the pre-determined depth identified in the project planning documents. For small vessels/floating platforms or above water stationary structures a pre-cleaned gravity corer is used. As with the hand corers/augers, the depth of sediment penetration into the sediment, together with sample handling procedures, is identified in the project planning documents. In some cases, corers may include a liner on the interior of the core tube. Sediment cores may be sectioned to provide vertical profiles of sediment characteristics.

Volatile organic and sulfide samples are collected immediately after sample retrieval, regardless of the sampling procedure used. If multiple samples are required to provide the sample volume identified in the project planning documents, then samples must be thoroughly homogenized prior to collection of aliquots for testing.

4 Cautions

This SOP is applicable to routine E & E aquatic sediment sampling and is limited to relatively shallow sediment sampling depths. Hand sampling is generally limited to the upper 10 cm of sediment. Grab sampling may extend to about 15 cm below the sediment surface. Corers used in this SOP are generally effective only to a maximum depth of 100 cm below the sediment surface. The depth of sample collection will be limited if bottom sediment is sandy, clayey or rocky.

Sampling for some projects, such as dredging activities, usually requires deeper sediment sample collection and more sophisticated equipment (e.g., box corers or vibracorers). These are not activities typically contracted to a driller or vibracore operator and sample collection procedures using such equipment should be described in project planning documents. Sample sectioning and sub-sampling procedures described for hand core samples also are applicable to cores collected by a contractor.

Van Veen and Ponar grab samplers are designed for use in soft sediments. Other types of sampling devices may be required for use in clayey, sandy or rocky environments. Corers may work better than grabs in clayey or sandy substrates. Bottom dredges (not routinely used by E & E) may be required for sampling rocky substrates.

Because the sampling devices specified within this SOP provide limited sample volumes, multiple samples may be required to meet project DQOs. Sample compositing and homogenization should be addressed in the project planning documents. Sample aliquots for volatile organics, sulfide, or similar analytes should be collected as soon as possible after collection and prior to homogenization. Field personnel must maintain an awareness of the sediment sample volume collected versus the volume required to meet program/project DQOs.

Maintaining sample integrity requires selecting a sediment sampler that meets the project DQOs. Carefully following procedures should minimize the disruption of the sediment structure and subsequent changes in physiochemical and biological characteristics.

In flowing water, sediment samples should be collected moving from downstream to upstream. At sites with known or suspected contamination, samples should be collected moving from least contaminated area to most contaminated area.

Re-use of equipment may be unavoidable given size and cost. Decontamination should be matched to DQOs.

Experience has shown that real-world conditions (e.g., variable bottom conditions such as the presence of rocks or wood waste) may lead to unacceptable sediment sample recoveries and multiple attempts to collect sediment samples will be required at some locations.

Standard measures, such as the use of disposable gloves, that meet project DQOs, should be used to avoid cross contamination of samples.

As with all intrusive sampling work, project planning should address the potential for encountering subsurface “utilities” and the measures to be taken to avoid problems in the field.

5 Equipment and Supplies

The equipment and supplies required for field work depend on the program/project DQOs. The following is a general list of equipment and supplies. A detailed list of equipment and supplies should be prepared based on the project planning documents. In general, the use of dedicated or disposal equipment is preferred but equipment may be re-used after thorough decontamination between sample locations (refer to E & E Sampling Equipment Decontamination SOP ENV 3.15).

- Stainless-steel or Teflon™ spoons, trowels, or scoops. Other construction material may be acceptable depending upon the program/project planning documents and DQOs;
- Stainless-steel mixing bowls. Other bowl construction material may be acceptable depending upon the program/project planning documents and DQOs;
- Ekman grab(s);
- Modified Van Veen or Ponar grab sampler(s);
- Hand-driven auger(s), split core sampler(s), and multistage core sampler(s);
- Liners and/or catchers for augers or core samplers as specified in the project planning documents;
- Gravity corer(s) with weights, cutting edges, core catchers, end caps;
- Pipe cutter(s), stainless steel knives(s);
- Winch with hydrowire (power supply, e.g., generator if necessary). A hand operated winch may be used;
- Nylon line;
- Siphon (short length of Teflon or inert tubing);
- Core extruder;
- Connectors (e.g., Brummell hooks or shackles); and

- Ancillary equipment and supplies (e.g., meter stick or tape measure, aluminum foil, plastic sheeting, disposable gloves).

Supporting equipment and supplies also may be required to address the following:

- Field logbooks and supplies (Refer to project planning documents and the E & E Field Activity Logbooks SOP DOC 2.1 for details)
- Decontamination equipment and supplies (Refer to project planning documents and E & E Sampling Equipment Decontamination SOP ENV 3.15for details)
- Sample containers, preservatives, and shipping equipment and supplies (Refer to project planning documents and the E & E Environmental Sample Handling, Packaging and Shipping SOP ENV 3.16 for details)
- Waste handling supplies (Refer to project planning documents and E & E Handling Investigation-Derived Wastes SOP ENV 3.26 for details)

6 Procedures

E & E staff will use the following procedures for completing sediment sampling:

- Review relevant project planning documents, e.g., work plan, sampling and analysis plan, quality assurance project plan, health and safety plan, etc.
- Select the sampling procedure(s) that meet project DQOs.
- Refer to the E & E Field Activity Logbooks SOP DOC 2.1 for guidance on the types of information that should be recorded for each sample.
- Refer to the E & E Environmental Sample Handling, Packaging and Shipping SOP ENV 3.16 for guidance on how samples should be labeled, packaged, and shipped.

6.1 Hand Sediment Sampling

The following procedures are used for collecting sediment samples using hand tools. Wetlands, lakes/ponds, low-flow streams, and (with a tide that meets project DQOs) marine intertidal sediment samples may be collected by hand.

- Excavate shallow sediment with pre-cleaned spoons, trowels, or scoops.
- Minimize sediment disturbance.
- Identify sample collection intervals in the project planning documents. In general, the maximum depth of sample collection is 10 cm or less, although deeper sampling may be possible if the matrix is sufficiently stable for an excavation to remain open.
- Sampling device components that come into contact with the sediment samples should be constructed of stainless steel or Teflon™. Other materials may be appropriate if they meet project DQOs.
- Collect sufficient sample volume to meet the DQOs identified in the project planning documents
- Place aliquots to be analyzed for volatile organic analytes and/or sulfides directly into sample containers (i.e., prior to homogenation).
- Empty hand-collected samples into a pre-cleaned stainless steel bowl (or other type as specified in the project planning documents)

- If multiple hand collected samples are necessary to collect adequate sample volume, they should all be combined in the bowl prior to homogenization
- Homogenize the sample(s) as thoroughly as possible
- Transfer sample aliquots to appropriate sample containers and preserve as required in the project planning documents.
- Return unused sediment to the excavation when sampling is complete.

6.2 Grab Sediment Sampling

The following procedures are used for collecting sediment samples using a stainless steel Ekman dredge. Other Ekman construction materials may be appropriate if they meet project DQOs.

- Clean the Ekman grab prior to use.
- Open and lock the grab jaws
- Slowly lower the grab into the sediment
- Using whatever trip mechanism is associated with the grab, close the jaws.
- Retrieve the grab
- Empty the grab into a stainless steel bowl (or other type as specified in the project planning documents)
- Immediately collect volatile organic analyte and sulfide samples.
- If multiple Ekman grabs are necessary to collect adequate sample volume, they should all be combined in the bowl prior to homogenization
- Homogenize the sample as thoroughly as possible
- Transfer sample aliquots to appropriate sample containers and preserve as required in the project planning documents.
- Return unused sediment to the water when sampling is complete if allowed in the project planning documents.

The following procedures are used for collecting sediment samples using a grab sampling device such as a Ponar grab sampler.

- Grab sampling may be conducted from small vessels/floating platforms or stationary structures (e.g., bridges) above water. Refer to project planning documents for guidance related to sampling from small vessels.
- A pre-cleaned, modified 0.1-m^2 stainless steel Van Veen grab sampler is the preferred grab sampler for routine sediment collection.
- Ponar grab samplers are similar in design and operation to Van Veen samplers and may also be used. The maximum depth of sediment penetration that can be expected is about 15 cm, less in clayey, sandy or rocky environments. Van Veen and Ponar grabs may not be the most appropriate sampling devices for such matrices.
- Open and lock the grab jaws
- Remove the safety pin only after the grab is clear of the sampling platform

- Slowly lower the grab using a power winch-hydrowire, or by hand line to avoid a pressure wave
- The speed of decent should be about 1-foot per second within 1 meter from the bottom
- Once the grab reaches the bottom, the sampler will be “tripped”.
- Raise the grab slowly to allow proper jaw closure.
- Retrieve the grab. Do not exceed a 4-foot per second ascent speed to avoid disturbing the sample.
- Secure the grab on the sampling platform
- Open the upper sample access door(s) and evaluate the sample for acceptability
- The following criteria must be met for the sample to be acceptable
    - Sampler jaws should be closed (no rocks, sticks, or other materials should be trapped in the jaws since this would allow for sample washout from the grab)
    - Sampler must not be overfilled (overfilling could result in sample loss)
    - Overlying water is present (indicating sample integrity)
    - Sediment surface appears relatively undisturbed
    - The sediment surface should be even and roughly parallel to the top of the grab
    - Desired sample depth was achieved (ideally at least 1 cm of sediment should remain at the bottom of the sampler after the upper layer(s) have ben sampled)
- Siphon off overlying water (turbid water may be allowed to settle for a short period)
- Immediately collect volatile organic analyte and sulfide samples.
- Depending on the project DQOs, the entire sample may be transferred to a stainless-steel mixing bowl for homogenization and collection of sample aliquots.
- The sediment within grab also may be subsampled. Avoid taking sediment that has come in direct contact with the grab sampler.
- Pre-cleaned stainless steel or Teflon™ spoons, spatulas, or other scoops may be used to collect sediment from within the grab. Other scoop construction materials may be appropriate if they meet project DQOs.
- Place sediment into stainless steel mixing bowl (or other type as specified in the project planning documents)
- If multiple Van Veen grabs (or subsections from within multiple grabs) are necessary to collect adequate sample volume, they should all be combined in the bowl prior to homogenization
- Homogenize the sample as thoroughly as possible
- Transfer sample aliquots to appropriate sample containers and preserve as required in the project planning documents.
- Return unused sediment to the water when sampling is complete if allowed in the project planning documents.

6.3 Core Sediment Sampling

The following procedures are used for collecting sediment samples using a sediment hand core. The subsampling and sectioning procedure also is applicable to core samples collected by a contractor with vibracore. Specific procedures for collection of vibracore samples by a contractor should be included in the project planning documents.

Manual core sediment sampling may be conducted in wetlands, lakes/ponds, low-flow streams, and with a tide that meets project DQOs, the marine intertidal zone. Mechanical core sediment sampling also may be conducted from small vessels/floating platforms or stationary structures (e.g., bridges) above water. Refer to project planning documents for guidance related to sampling from small vessels. Core sampling is recommended if accurate resolution of sample depths is a DQO

There are a variety of manual sediment core sampling devices available for collecting virtually undisturbed sediment core samples. Augers, split core samplers, and multistage core samplers may be used with or without liners that are used to avoid contact between the sediment and the corer. While there are many types of mechanical coring devices, E & E routinely uses only gravity corers. Gravity corers may or may not include a liner.

The following procedures are used for collecting sediment samples using a coring device.

- Pre-clean the coring equipment. See E & E Sampling Equipment Decontamination SOP ENV 3.15 for decontamination procedures.
- Before deployment, visually inspect the sediment retainer (core catcher) to verify the seal should be sufficient to prevent loss of core sediment.
- If hand coring drive the pre-cleaned manual corer into the sediment and retrieve by hand. Hand coring will generally be limited to 2-inch diameter - 1 meter long samples.
- If using a winch from a sampling platform modify the procedure as follows:
    - Adjust the depth of penetration by adding or removing weights from the top of the corer.
    - Slowly lower the corer using a power winch to prevent the core tube from swinging. The corer should enter the bottom vertically.
    - The corer should be allowed to free-fall from 5 to 10 meters above the bottom
    - Once the corer has penetrated the sediment (based on visual changes in wire strain), the winch should be braked.
    - Use the winch to extract the corer (Considerable strain on the hydrowire can occur when a core tube is embedded in sediment. Use a steady continuous pull to lift the coring device). Do not exceed a 4-foot per second ascent speed to avoid disturbing the sample.
- Bring the corer out of the water and place onto the shore or sampling platform.
- Note if there is sample leakage at the cutter end.
- Sediment cores should be capped and stored upright if not sampled immediately. In general though cores should be split as soon as possible following collection.
- After allowing the surface sediment to settle, siphon off the surface water from the top of the core tube.

- Evaluate compaction (core length versus depth of penetration [based on sediment traces on the outside of the core tube]).
- The following criteria must be met for the sample to be acceptable.
  - Core catcher should be closed (no rocks, sticks, or other materials should be trapped in the catcher)
  - Core tube must not be overfilled (overfilling could result in sample loss)
  - Overlying water is present (indicating sample integrity)
  - Desired sample depth was achieved
- Sediment cores should be extruded or split as soon as possible following collection.
  - Decant water from the top of core barrel or drill a small opening above the sediment line to allow the surface water to drain.
  - Place core barrel or liner on clean surface
  - Carefully remove end caps or catchers
  - For transverse sectioning, beginning at the sediment surface, measure and mark the sample sections on the outside of the liner
    - Cut the liner with a manual pipe cutter or core liner and core with a decontaminated saw blade into marked sections.
    - Extrude the sediment from the cut segments of the liner. If necessary use a plunger cover with aluminum foil to aid in extruding the core.
    - For some geotechnical sampling the sediment may need to remain in the core liner and be cap and sealed.
    - Empty the core segment into a stainless steel bowl (or other type as specified in the project planning documents).
    - Record observations of the sediment types.
    - Immediately collect volatile organic analyte and sulfide samples.
  - For longitudinal sectioning, open the split tube or use a knife to cut the liner and expose the upper half of the sediment cylinder.
    - Beginning at the sediment surface, measure and mark the sample sections using a tape measure set aside the core.
    - Record observations of the sediment types.
    - Immediately collect volatile organic analyte and sulfide samples.
    - Scope the core segment into a stainless steel bowl (or other type as specified in the project planning documents).
- If multiple core segments are necessary to collect adequate sample volume, they should all be combined in the bowl prior to homogenization.
- Homogenize the sample as thoroughly as possible.
- Decant any excess water. Sediment samples should be have greater than 30% solids and greater than 50% is preferred.

- Transfer sample aliquots to appropriate sample containers and preserve as required in the project planning documents.
- Return unused sediment to the water when sampling is complete if allowed in the project planning documents.

In very shallow water and soft sediment, the coring procedure can be modified to use only the core liner as follows. This procedure is only recommended for composite samples of the entire sediment depth.

- Drive the core liner into an undisturbed sediment area by hand.
- Once the liner is driven into the sediment, surface water is added to the top of the liner to create suction.
- Pull the core liner out of the sediment and place in the stainless steel bowl.
- Measure the sediment length and gently decant the water from the top of liner while holding the liner over the bowl.
- Once the suction is release the sediment should extrude into the bowl.
- Homogenize and sample as described above.

7 Quality Assurance/Quality Control

Prior to initiating field work, the project planning documents should be reviewed by field personnel to identify sampling procedure(s) that will most likely provide sediment samples that meet project DQOs.

The program/project manager should identify personnel for the field team who have knowledge, training and experience in the field sediment sampling activities being conducted. One member of the field team should be designated as the lead for sediment sampling and will be responsible, with support from other field personnel, for implementing the procedures in this SOP. The program/project manager should also identify additional personnel, if necessary, to complete ancillary procedures (e.g., field logbook documentation, equipment decontamination, sample shipment, and waste disposal).

The sediment sampling lead should prepare a detailed equipment checklist before entering the field and verify that sufficient and appropriate equipment and supplies are taken into the field.

Guidelines for accepting a sediment grab or core are noted within the sampling procedures. Unacceptable samples should be discarded.

Volatile organic analyte and sulfide samples should always be collected prior to homogenization.

Quality assurance/quality control samples (e.g., co-located samples) are collected according to the site quality assurance project plan. Field duplicates are collected from one location and treated as separate samples. Field duplicates are typically collected after the samples have been homogenized. Collocated samples are generally collected from nearby locations and are collected as completely separate samples.

In cases where multiple hand-collected samples; grabs; or cores are required to generate an adequate sample volume, homogenization is important. Field personnel should collect sample aliquots only after mixing has produced sediment with textural and color homogeneity.

In flowing water, sediment samples should be collected moving from downstream to upstream. At sites with known or suspected contamination, samples should be collected moving from least contaminated area to most contaminated area.

8 Health and Safety

Prior to entering the field, all field personnel should formally acknowledge that they have read and understand the project specific health and safety plan.

Hazards associated with wetlands work should be clear (e.g., engulfment and snakes) and proper precautions noted.

Ekman, Van Veen, and Ponar sampling apparatus are inherently dangerous pieces of heavy equipment which a high “pinch” potential. Care should be taken at all times when handling such equipment, not just during sample collection.

Grab samplers and coring devices are difficult to handle on small vessel decks and floating platforms. Care should be taken whenever handing heavy equipment. Be sure sampling devices are well-secured when not in active use.

Hazardous preservatives (e.g., acids, solvents, and formalin) should be properly handled and stored.

Work aboard small vessels and floating platforms should conform to good safe boating practices, coast guard (or other competent authority) guidance/regulations, and the boat operators standard operating procedures.

9 Special Project Requirements

Project or program-specific requirements that modify this procedure should be entered in this section and included with the project planning documents.

10 References

The following list sources of technical information on sediment sampling.

American Society for Testing and Materials, 1993, ASTM Standards on Aquatic Toxicology and Hazard Evaluation, Philadelphia, PA, ASTM Publication Code Number (PCN): 03-547093-16.

British Columbia Field Sampling Manual for Continuous Monitoring and the Collection of Air, Air-Emission, Water, Wastewater, Soil, Sediment, and Biological Samples, 2003 Edition, Prepared and published by: Water, Air and Climate Change Branch Ministry of Water, Land and Air Protection Province of British Columbia, January 2003

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Commonwealth of Virginia, Department of Environmental Quality, November 2010, Standard Operating Procedures Manual for the Department of Environmental Quality Water Monitoring and Assessment Program, Revision No. 18.

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Great Lakes National Program Office, 1985, Methods Manual for Bottom Sediment Sample Collection, United States Environmental Protection Agency, Chicago, Illinois, EPA-905/5-85-004.

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Manual for Collection, Storage and Manipulation of Sediments for Chemical and Toxicological Analyses: Technical Manual, U.S. EPA, Office of Water, EPA-823-B-01-002, October 2001

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Navy Environmental Compliance Sampling and Field Testing Procedures Manual, NAVSEA T0300-AZ-PRO-010

Pacific Northwest Laboratories, 1987, Guidance for Sampling of and Analyzing for Organic Contaminants in Sediments, United States Environmental Protection Agency, Criteria and Standards Division, Richland, Washington.

QA/QC Guidance for Sampling and Analysis of Sediments, Water, and Tissues for Dredged Material Evaluations Chemical Evaluations, U.S. EPA, Office of Water, EPA-823-B-95-001, April 1995

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United Nations Environment Programme, Mediterranean Action Plan, Manual on sediment Sampling and Analysis, UNEP(DEPI)/MED WG.321/Inf.4, 7 November 2007

United States Department of Commerce, National Technical Information Service, 1985, Sediment Sampling Quality Assurance User's Guide, Nevada University, Las Vegas, NV.

END OF SOP

ecology and environment, inc.

STANDARD OPERATING PROCEDURE

SAMPLING EQUIPMENT DECONTAMINATION

SOP NUMBER: ENV 3.15

REVISION DATE: 5/25/2012 **SCHEDULED REVIEW DATE: 5/25/2017**

Contents

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1 Scope and Application

This Standard Operating Procedure (SOP) describes the routine procedures utilized by E & E personnel in the field for decontaminating sampling equipment that is not dedicated or disposal and that may have come into contact with site contaminants. It is applicable for equipment that will be re-used in the field and for equipment that will be returned to a warehouse or other storage facility prior to re-use.

Program/project specific data quality objectives (DQOs) dictate the types of sampling equipment requiring decontamination and site-specific sampling procedures should be identified in program/project planning documents. This SOP applies to equipment routinely used for:

- Water quality sampling (e.g., buckets, bailers, Kemmerers, and Niskins);
- Flow/water depth measuring (e.g., velocity meters, stream gauges, and depth sounders);
- Soil and sediment sampling (e.g., corers, augers, Van Veens, direct-push samplers, homogenization buckets, and mixing tools); and
- Miscellaneous tools (e.g., shovels, scoops, tapes/rulers/meter sticks, and cutting tools).

Decontamination is time consuming and expensive, often including analyses of field rinsates and other "blanks" to verify decontamination procedures provide equipment that meet program/project DQOs. The use of clean, dedicated, disposable equipment (e.g., Teflon or plastic bailers for groundwater sampling, aluminum bowls for soil homogenization) is preferred, whenever practicable.

This sampling equipment decontamination SOP is intended for use by personnel who have knowledge, training, and experience in the field sampling activities being conducted and who understand the importance of decontamination in meeting program/project-specific DQOs.

The SOP does not address personnel decontamination. As part of the health and safety plan, a personnel decontamination plan should be developed and set up before any personnel or equipment enters the areas of potential contamination.

2 Definitions and Acronyms

ASTM	American Society for Testing and Materials
De-ionized water	Purified water produced by distillation or by filtration through de-ionizing columns or other means (e.g., reverse osmosis) or some combination of treatments. Program/project DQOs establish the level of purity required (e.g., maximum level of electrical conductivity)
DQO	Data quality objective
Potable water	Tap water from a treated drinking water supply
SHASP	Site-specific Health and Safety Plan
SOP	Standard Operating Procedure
USEPA	United States Environmental Protection Agency

3 Procedure Summary

Sampling equipment decontamination procedures vary depending on the DQOs identified in the program/project planning documents . These documents address the types and degrees of contamination anticipated and identify appropriate decontamination procedures, materials, and wastes handling.

A decontamination line is set up in the contamination reduction zone, outside of the contamination "hot" zone, where personnel follow a multi-step decontamination procedure. If a formal decontamination line is established for the site, then all equipment decontamination must be completed with the "hot" zone.

This procedure can be expanded to include additional or alternate wash/rinse steps designed to remove specific target analytes/compounds, if required by site-specific work plans or as directed by a particular client.

4 Cautions

Decontamination of sampling equipment left in situ for long periods (e.g., groundwater pumps, stack samplers, continuous flow samplers) is addressed in program/project-specific planning documents.

Sites with biohazards are not considered routine operations. Biohazard site sampling equipment decontamination is addressed site-specific program/project planning documents.

Sites with explosive hazards are not considered routine operations. Explosives site sampling equipment decontamination is addressed in site-specific program/project planning documents.

Sites requiring ultra-clean sampling methods (e.g., United States Environmental Protection Agency [USEPA] Method 1669) require ultra-clean sampling equipment decontamination. Ultra-clean sampling equipment decontamination is addressed in site-specific program/project planning documents.

Decontamination of contaminated or potentially contaminated sampling equipment may generate incompatible hazardous wastes. Only compatible waste streams, as defined in the program/project planning documents are combined for disposal.

The use of distilled/deionized water commonly available from commercial vendors may be acceptable for decontamination of sampling equipment provided that it has been verified by laboratory analysis to be analyte-free distilled/deionized water. Analyte-free deionized water is can be obtained from the project analytical laboratories if available. Distilled water available from local grocery stores and pharmacies is generally not acceptable for final decontamination rinses. Contaminant-free deionized water is that has been stored on site should not be used without testing. Any new source of water should be tested prior to use if not certified by a vendor or laboratory.

In general, use of solvents is avoided for low level environmental analysis, but may be necessary for more contaminated areas.

5 Equipment and Supplies

Planning documents provide direction on the specific equipment and supplies, and the numbers/volumes required to meet program/project-specific DQOs. The following equipment and supplies are used for routine sampling equipment decontamination:

- Appropriate protective clothing (including safety glasses or splash shield and nitrile gloves);
- Galvanized or similar wash basins;
- Waste collection drums (if required) ;
- Plastic buckets (5-gallon);
- Long-handled brushes;
- Spray/squeeze bottles;
- Non-phosphate detergent (e.g., Liquinox™ or Alconox™);
- Pesticide grade (or equivalent) organic solvents (e.g., methanol, hexane, or other as specified in the planning documents.) if necessary based on the contaminants
- Ten percent, by volume in de-ionized water, nitric acid (ultrapure);
- Tap water;
- Deionized water (usually American Society for Testing and Materials [ASTM] Type II);
- Organic-free water;
- Plastic sheeting for ground cover;
- Paper towels;
- Trash bags;
- Aluminum foil; and
- Waste handling supplies. (Refer to project planning documents and E & E Investigation-Derived Waste SOP for details.)

Note all waters, acids and detergents should be are stored in their original containers or clearly marked clean sealable glass, plastic, or Teflon® bottles in which information from the original label has been transferred. The secondary labeling should include reagent name, source, date opened/transferred, and expiration date as well as any hazardous labels.

6 Procedures

Before entering the field personnel reviews relevant program/project planning documents (e.g., work plan, sampling and analysis plan, quality assurance project plan, health and safety plan);and select the sampling equipment decontamination procedures (e.g., organic solvent[s] to be used) that meet project DQOs.

In the field personnel should follow best practices to minimize contamination of equipment and prevent cross contamination of cleaned equipment.

- Set-up a zone that isolates areas of contamination from clean areas of the site. All equipment should be decontaminated within the contamination area.
- Employing work practices that minimize contact with hazardous or toxic substances (e.g., avoid areas of obvious contamination, avoid touching potentially contaminated materials);
- Covering monitoring and sampling equipment with plastic or other protective material;

- Use of disposable outer garments and disposable sampling equipment with proper containment of these disposable items;
- Use of disposable towels to clean the outer surfaces of sample bottles before and after sample collection; and
- Encasing the source of contaminants with plastic sheeting or overpacks.

6.1 Decontamination Methods for Direct Sample Contact Equipment

Field personnel should set-up a decontamination line that moves contaminated equipment through the decontamination process to a clean zone. At all stations in the decontamination line, contaminated and/or potentially contaminated fluids and/or wastes are collected and containerized.

Routine decontamination steps for equipment that directly contacts samples are described below.

1. Physically remove gross contamination from equipment by abrasive scraping and/or brushing.
2. Wash equipment with non-phosphate detergent (i.e., Alconox™ or Liquinox™) in tap water.
3. Rinse with tap water
4. Rinse with de-ionized water.
5. Rinse with 10% nitric acid, if specified in planning documents. Nitric acid washes are typically used for metals contamination.
6. Rinse with de-ionized water (if the acid rinse is conducted).
7. Rinse with organic solvent(s) to remove high levels of organic contamination, refer to the planning documents for the site/activity-specific solvent choice.

   Use a methanol rinse to dissolve and remove soluble organic contaminants for high concentration samples.

   Use a hexane rinse to dissolve waste lubricating oils, tars, and bunker fuels for high concentration samples.
8. Air drying
9. Rinse with deionized, organic-free water, usually only if alternative solvents are used.
10. Wrap sampling equipment in aluminum foil or plastic ; if it will not be used immediately. Determine the best material to wrap equipment based on site contaminants for example plastic bags should not be used is sampling for volatile and extractable organics.
11. Containerize all solvent rinsing wastes, detergent wastes and other chemical wastes requiring off-site or regulated disposal. Dispose of all wastes in conformance with applicable regulations as defined in the project planning documents.

6.2 Decontamination Methods for Other Equipment and Meters

Several types of sampling equipment such as meters, pumps and tubing that cannot be cleaned directly as described in 6.1. Consult the manufacturers guidelines before decontaminating and equipment.

General decontamination steps are described below.

1. Physically remove visible contamination from equipment by brushing the outside of the equipment or wiping with paper towel.
2. If tubing or other portions of the equipment comes into contact with the sample then pump any decontamination solvents through the equipment.
3. Rinse/or pump with tap water
4. Rinse/or pump with de-ionized water.
5. Air dry
6. Wrap sampling equipment in aluminum foil or plastic ; if it will not be used immediately. Determine the best material to wrap equipment based on site contaminants.

6.3 Decontamination Methods for Heavy Equipment

For heavy equipment, a decontamination pad should be established by the driller or subcontractor. Heavy sampling equipment (e.g., augers) decontamination may include a steam cleaning and/or high-pressure water wash step after gross contamination is removed by detergent and brushing.

7 Quality Assurance/Quality Control

Program/project planning documents define the quality assurance/quality control procedures (e.g., collection and analysis of equipment rinsate and other “blanks”) necessary to meet program/project DQOs. Typically, a field blank (equipment rinsate blank) consists of a sample of analyte-free water passed through/over a decontaminated sampling device to assess possible cross contamination from equipment to sample contamination.

8 Health and Safety

Personnel review and acknowledge that they understand the project planning documents, especially the SHASP prior to entering the field. Material Safety Data Sheets are taken into the field for hazardous materials used at a site.

Some types of sampling equipment are inherently dangerous pieces of heavy equipment with high pinch or crush potential. Proper handling procedures are followed during decontamination of heavy equipment.

Decontamination procedures may pose hazards, especially when chemical decontamination procedures, high pressure, and/or steam are used. Exposure to hazardous materials or wastes is controlled by the use of appropriate personal protective equipment and proper handling and storage of the materials/wastes, as specified in the project planning documents, especially the SHASP.

Steam cleaning - follow equipment manufacturer operating and safety guidelines.

High-pressure water cleaning - follow equipment manufacturer operating and safety guidelines.

Waste collection and disposal procedures are presented in program/project planning documents and E & E Investigation-Derived Waste SOP.

Avoiding practices that increase tendencies for hand-to-mouth contact including: eating, drinking, smoking, or using chewing tobacco is a basic procedure employed during all field activities.

9 Special Project Requirements

Special project requirements are presented in the program/project planning documents. If required, contract or other client-specific, site-specific requirements may be entered in this section.

10 References

The following list sources of technical information on decontamination procedures.

ASTM D 5088 – 02 Standard Practice for Decontamination of Field Equipment Used at Waste Sites, 2008

USEPA Environmental Response Team “Sampling Equipment Decontamination”, SOP #: 2006, REV.#:0.0, 08/11/94

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Navy Environmental Compliance Sampling and Field Testing Procedures Manual, NAVSEA T0300-AZ-PRO-010

END OF SOP

ecology and environment, inc.

STANDARD OPERATING PROCEDURE

ENVIRONMENTAL SAMPLE HANDLING, PACKAGING AND SHIPPING

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Contents

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1 Scope and Application

This Standard Operating Procedure (SOP) describes the packing, marking, labeling, and shipping procedures routinely used by E & E field personnel to transfer environmental samples from the field to off-site laboratories. Unpreserved and/or properly preserved environmental samples include the following matrices:

- Drinking water;
- Groundwater;
- Surface water;
- Soil;
- Sediment;
- Treated municipal and industrial effluent;
- Biological specimens (i.e., non-pathogenic plant and/or animal tissue); or
- Samples not expected to be contaminated with high levels of hazardous substances.

Shipping includes transport by air, rail, or motor vehicle.

Samples containing known or suspected International Air Transport Authority (IATA)-defined dangerous goods and/or United States Department of Transportation (DOT)-defined hazardous materials or which have anesthetic, noxious, or other properties that could inhibit the abilities of transporters do not meet the criteria for shipping as "environmental" samples.

This environmental sample packaging and shipping SOP is intended for use by personnel who have knowledge, training, and experience in the procedures described herein and who have received training on E & E's On-line Hazardous Materials/Dangerous Goods Shipping Guidance Manual. Regional Hazardous Materials Transportation Coordinators (RHTCs) are available to provide technical support for environmental sample shipping.

In the event the sample material meets the established criteria of a DOT hazardous material, consult one of the RHTC personnel and follow guidelines in E & E's Hazardous Materials/Dangerous Goods Shipping Guidance Manual (see http://www.corp.ene.com/departments/health_&_safety/shipping_manual.asp).

2 Definitions and Acronyms

^{o}C	degrees Celsius
COC	Chain-of-Custody
DNAPL	dense non-aqueous phase liquid
DOT	(United States) Department of Transportation
DQO	Data Quality Objective
EPA	United States Environmental Protection Agency
IATA	International Air Transport Authority
LNAPL	light non-aqueous phase liquid
RHTC	Regional Hazardous Materials Transportation Coordinator

SHASP	Site-specific Health and Safety Plan
SOP	Standard Operating Procedure
UN	United Nations
VOA	volatile organic analysis

3 Procedure Summary

Sample packaging, marking, labeling and shipping procedures vary depending on the data quality objectives (DQOs) identified in the program/project planning documents (e.g., work plan, sampling and analysis plan, quality assurance project plan, SOPs, and site-specific health and safety plan [SHASP]). These documents address the types and degrees of contamination anticipated and identify appropriate shipping and handling procedures.

Properly identified, preserved, and sealed individual sample bottles/jars provided by field samplers are sealed in plastic bags and placed in lined shipping containers. Packing material (e.g., bubble wrap) is used reduce the risk of damage to sample bottles/jars and loss of samples during transport. Absorbent material (e.g., highly absorbent small animal bedding material made from recycled paper/wood waste) is added to the shipping container to contain spills from sample bottles/jars during transport. Double-bagged ice is added to the shipping containers as a preservative. Chain-of-custody (COC) documents are prepared and enclosed in the shipping containers. Shipping containers are marked in compliance with DOT/IATA regulations. Shipping papers (e.g., Federal Express shipping documents) are completed and attached to the shipping containers. Shipping containers are custody sealed and taped. Clients, program/project managers, shippers and laboratories already scheduled to receive samples are notified daily of impending shipments.

4 Cautions

Samples collected from sources, such as waste lagoons, drums, tanks, heavily stained soils, and groundwater contaminated with LNAPL or DNAPL, do not qualify as environmental samples.

Known or suspected samples of IATA-defined dangerous goods and/or DOT-defined hazardous materials do not meet the criteria for shipping as “environmental” samples.

Shipping of IATA dangerous goods and/or DOT hazardous materials is not covered by this SOP. Guidance on shipping dangerous goods and hazardous materials is presented in E & E’s Hazardous Materials/Dangerous Goods Shipping Guidance Manual (see http://www.corp.ene.com/departments/health_&_safety/shipping_manual.asp).

Samples preserved in accordance with United States Environmental Protection Agency (EPA) Contract Laboratory Program guidance (most current version) are routinely shipped as environmental samples.

A RHTC should be consulted prior to any biological specimen shipping.

Transboundary/International shipping requirements are presented in program/project planning documents.

Samples preserved with methanol are not shipped as environmental samples. DOT/IATA regulations apply to the shipment of methanol preserved samples.

Individual sample bottle/jar labels are the responsibility of the field samplers who verify that labels are complete and correct, and match the COC forms prior to shipment to laboratories.

Known or suspected PCB and dioxin samples require additional packaging (i.e., sealing in metal cans) and are not covered by this environmental sample packaging and shipping SOP.

It is E & E's intent to package samples so securely to prevent leakage during shipment. This is to prevent the loss of samples and the expenditure of funds for emergency responses to spills and the efforts necessary to re-obtain the sample. Liquid samples are particularly vulnerable. Because transporters (carriers) are not able to know the difference between a package leaking distilled water and a package leaking a hazardous chemical, they will react to a spill in an emergency fashion, potentially causing enormous expense to E & E for the cleanup of the sample material. Therefore, liquids are to be packed in plastic bags and absorbent/cushioning material to help prevent possibility of leaks from a package.

5 Equipment and Supplies

Coolers, sample bottles/jars, COC forms, and sample labels are typically supplied by the laboratory.

Federal Express or other shippers provide shipping forms.

Packaging material, such as plastic bags, ice, and absorbent material, are purchased locally.

E & E-purchased durable packaging equipment, such as coolers, are labeled with the applicable E & E office (or, in some cases, field office) address.

6 Procedure

6.1 Prior to Field Activity

- Program/project managers or designated personnel utilize the project planning documents to stage the equipment and supplies required to meet project DQOs.
- Labeled temperature blanks, tap water filled 40-mL volatile organic analysis (VOA) vials, are prepared for use in the field.
- The project manager or designee arranges for shipper support and coordinates with the laboratory(ies) necessary to conduct the tests needed to meet project DQOs.

6.2 Field Sampler Support

Field samplers collect samples in accordance with the program/project planning documents and provide properly identified, preserved, and sealed individual sample bottles/jars to the field personnel responsible for sample packaging, marking, labeling, and shipping.

6.3 Environmental Sample Packaging Procedures

Environmental samples are usually shipped in 80-quart solid outer shell plastic or metal coolers (although other size coolers may be used if they meet program/project needs). Disposable, pressed Styrofoam coolers are not used. Before use, shipping cooler drain holes are sealed to prevent leakage. Non-applicable labels are removed from the cooler. Marking, Labeling, and Shipping procedures are presented in Section 6.4 of this SOP.

The following steps are used for routine packaging:

- Verify that the bottle is clean and labeled;
- Verify the caps are secure cap and if necessary use fiber reinforced tape;

- Seal each sample bottle and temperature blank in a sealable plastic bag; and
- Add one temperature blank to each cooler.

When a precut foam block insert is used to prevent sample bottle breakage during shipping:

- Verify cooler has this side up labels/arrows;
- Place at least 1 inch of inert absorbent material in the bottom of the cooler;
- Line the cooler with two double-bagged plastic (e.g., large heavy-duty garbage) bags;
- Place a foam insert (with holes cut to receive the sample bottles) inside the plastic bag;
- Place the bottles upright in the holes in the foam block;
- Fill void spaces with double-bagged ice to the top of the cooler;
- Seal each plastic bag lining the cooler with tape;
- Place a COC form in a waterproof, sealable bag taped to the inside of the cooler lid;
- Place custody seals over top edge of cooler so cooler cannot be opened without braking seals;
- Cover the custody seals with clear tape; and
- Secure the cooler with strapping tape over the hinges and around the entire cooler.

When bubble wrap or similar packing is used to prevent sample bottle breakage during shipping:

- Verify cooler has this side up labels/arrows,
- Place at least 1 inch of inert absorbent material in the bottom of the cooler,
- Line the cooler with two double-bagged plastic (e.g., large heavy-duty garbage) bags,
- Surround each bottle/jar (including the bottom) with bubble wrap, taping the wrap securely around the bottle,
- Place the bottles upright in the inner bag,
- Fill void spaces with double-bagged ice to the top of the cooler,
- Seal each plastic bag lining the cooler with tape,
- Place a COC form in a waterproof, sealable bag taped to the inside of the cooler lid, and
- Place custody seals over top edge of cooler so cooler cannot be opened without breaking seals;
- Cover the custody seals with clear tape; and
- Secure the cooler with strapping tape over the hinges and around the entire cooler.

When only absorbent material is used to prevent sample bottle breakage during shipping:

- Place at least 1 inch of inert absorbent material in the bottom of the cooler;
- Line the cooler with two double-bagged plastic (e.g., large heavy-duty garbage) bags;
- Place at least 1 inch of inert absorbent material in the bottom of the inner bag;

- Place each sample bottle upright inside the inner bag maintaining at least 3 inches between bottles;
- Fill the void spaces around the bottles with absorbent to at least half the height of the largest bottles;
- Fill void spaces with double-bagged ice to the top of the cooler;
- Seal each plastic bag lining the cooler with tape;
- Place a COC form in a waterproof, sealable bag taped to the inside of the cooler lid;
- Place custody seals over top edge of cooler so the cooler cannot be opened without braking the seals;
- Cover the custody seals with clear tape; and
- Secure the cooler with strapping tape over the hinges and around the entire cooler.

6.4 Marking, Labeling and Shipping Procedures

Program/project planning documents provide the information necessary to initiate filling out the COC forms. Additional information is available in the site field logbook(s).

Environmental samples are shipped as nonhazardous cargo.

Outer marking and labeling on each container is compliant with requirements for the carrier that will be used requirements. Coolers have this side up or arrow labels affixed. Extraneous markings are removed.

Markings indicating ownership of the container, destination, and shipping company labels are acceptable and attached as required.

Hazardous materials/dangerous goods airbills are not used when shipping environmental samples.

Environmental sample packages generally shipped overnight by Federal Express or equivalent. Field personnel check with shippers in advance to verify both pick-up and delivery schedules; especially when weekend and/or holiday pick-up and/or delivery may be required.

7 Quality Assurance/Quality Control

Hazardous Materials/Dangerous Goods Shipping training is provided to personnel responsible for shipping environmental samples. RHTCs are available to provide technical support for environmental sample shipping.

COC forms may be completed electronically or by hand. Samples recorded on the COC form are checked against the packaged samples.

Custody seals are attached to shipping containers so the receiving laboratory may verify the temperature of the samples.

Field samplers and shipping personnel verify the samples in the cooler and the samples listed on the COC match.

Site-identifying information is not listed on samples, forms, or other documents and is not provided to the receiving laboratory(ies).

Clients, program/project managers, shippers, and laboratories already scheduled to receive samples are notified daily of impending shipments. E & E personnel verify shipping addresses and confirm the receiving facility's commitment to accept samples based on shipment dates.

Samples shipped on ice require preservation to 4°C (±2°C). Samples that arrived at the laboratory outside this range could have compromised data quality. Samples should be cooled prior to packaging and sufficient ice used to keep samples cool particularly in warm weather. If samples are being shipped for Saturday or holiday delivery, then the availability of personnel should be verified with the laboratory and the shipping documentation checked to verify the appropriate delivery date is noted. Always confirm delivery of the samples with the shipper.

8 Health and Safety

Prior to entering the field, personnel will formally acknowledge that they have read and understand the project specific health and safety plan (SHASP).

Preserved samples (e.g., samples containing acids, solvents, and formalin) will be handled in accordance with the SHASP.

Good basic lifting and handling procedures will be followed when handling filled coolers.

9 Special Project Requirements

Special project requirements may be found in the program/project planning documents.

10 References

U.S. Environmental Protection Agency (EPA). 2011. Office of Superfund Remediation and Technology Innovation, *Contract Laboratory Program Guidance for Field Samplers*, OSWER 9240.0-47 EPA 540-R-09-03, January 2011. Accessed online at: http://www.epa.gov/superfund/programs/clp/download/sampler/CLPSamp-01-2011.pdf.

END OF SOP

ecology and environment, inc.

STANDARD OPERATING PROCEDURE

HANDLING INVESTIGATION-DERIVED WASTES

SOP NUMBER: ENV 3.26

REVISION DATE: 5/25/2012 **SCHEDULED REVIEW DATE: 5/25/2017**

Contents

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1 Scope and Application

This Standard Operating Procedure (SOP) describes the procedures utilized by E & E for handling of investigation derived wastes (IDW). Procedures for handling hazardous and non-hazardous categories of IDW are provided. Both categories of IDW may include:

- Solid wastes (e.g., soil cuttings, drilling muds);
- Liquid wastes (e.g., purged groundwater, decontamination fluids); and
- Personal protective equipment (e.g., coveralls, gloves).

This SOP provides references to applicable regulatory standards. In all cases the current applicable regulatory standards should be followed.

This SOP does not apply to IDW associated with explosives, radioactive, mixed, or pathogenic wastes or to projects outside of the United States. These IDW procedures should be identified in program/project planning documents. IDW procedures also vary depending on the site contaminants, site conditions, sampling procedures and applicable standards. These site-specific IDW procedures should be identified in program/project planning documents.

This SOP for handling IDW is intended for use by personnel who have knowledge, training, and experience in field activities that generate IDW and how that IDW is handled.

2 Definitions and Acronyms

CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CFR	Code of Federal Regulations
CWA	Clean Water Act
DOT	U.S. Department of Transportation
Hazardous Waste	The U.S. EPA defines a hazardous waste as “Hazardous waste is waste that is dangerous or potentially harmful to our health or the environment. Hazardous wastes can be liquids, solids, gases, or sludges. They can be discarded commercial products, like cleaning fluids or pesticides, or the by-products of manufacturing processes.” http://www.epa.gov/osw/hazard/
IDW	Investigation-Derived Wastes are solid and/or liquid wastes resulting from field activities
Non-hazardous Waste	Wastes that are not listed hazardous wastes, do not exhibit hazardous waste characteristics, are not universal wastes or which are not otherwise classified as hazardous
PPE	Personal Protective Equipment
RCRA	Resource Conservation and Recovery Act
SOP	Standard Operating Procedure
TDU	Treatment/disposal unit
TSCA	Toxic Substances Control Act

3 Procedure Summary

Waste minimization is the most critical component of IDW management.

Program/project planning documents (e.g., work plans, sampling and analysis plans, quality assurance project plans, and site-specific health and safety plans) should identify the types of IDW anticipated during field work, along with anticipated waste volumes and opportunities for on-site treatment, storage, and disposal.

Hazardous and non-hazardous wastes should be handled separately. Hazardous wastes may require separate sampling and analysis and later disposal activities. Resource Conservation and Recovery Act (RCRA) regulations place restrictions on the handling of hazardous wastes. These regulations may be viewed by clicking on the following link:

http://www.epa.gov/osw/hazard/wastetypes/wasteid/char/hw-char.pdf

U.S. Department of Transportation (DOT) regulations (49 Code of Federal Regulations [CFR] Parts 100-185) may also be applicable or relevant and appropriate requirements for handling IDW taken off-site. Regulations may be viewed by clicking on the following link:

http://www.fmcsa.dot.gov/safety-security/hazmat/complyhmregs.htm#hm.

There may also be other federal (e.g., Comprehensive Environmental Response, Compensation and Liability Act [CERCLA], Toxic Substances Control Act [TSCA], and Clean Water Act [CWA]), state, and/or local government regulations apply to IDW handling. Regulations or rules identified by other nations that including indian tribes are not included in this SOP and should be developed on a site-specific basis.

Site wastes, such as soil cuttings and purge water, whose characteristics are unknown are considered to be non-hazardous unless information is provided or field conditions indicate that such wastes are RCRA hazardous.

4 Cautions

This SOP does not apply to IDW associated with explosives, radioactive, mixed, or pathogenic wastes. Handling of these wastes should be addressed in program/project planning documents. IDW that poses specific toxicity or other safety (e.g., flammability) concerns should be addressed in program/project planning documents. Field personnel should verify that the site-safety plan addresses safe IDW handling.

RCRA regulations impose limitations and restrictions on the transport, storage, and disposal of hazardous wastes.

Record keeping for hazardous wastes should be in compliance with applicable or relevant and appropriate requirements.

Best professional judgment combined with knowledge of historical site activities and existing site processes/raw materials/waste streams information should be the first source of data in the characterization IDW. Sampling and analysis of wastes is both time consuming, expensive, and should be used only when necessary

It is important to ensure that all IDW is identified and accounted for, and if necessary containerized and properly labeled. A record should be kept of the number of drums/contents, etc., to be used later for sampling and waste disposal. Caution should also be taken when staging IDW, particularly when moving heavy containers. Additionally, proper PPE should be used, especially if the container contents are unknown.

5 Equipment and Supplies

Equipment needed for handling IDW includes:

- Fifty-five-gallon drums;
- Five-gallon buckets;
- Labels for drums and buckets;
- Wrench and hammer to secure drum lids;
- Lumber, plastic sheeting, and plywood for creating a temporary storage area;
- Trash bags;
- Sample containers;
- Indelible ink pen/permanent marker for labeling drums/containers;
- Manifests/shipping documents; and
- Plastic trash bags.

6 Procedures

6.1 Planning

Review relevant project planning documents (e.g., work plan, sampling and analysis plan, quality assurance project plan, and site-specific health and safety plan) for site-specific IDW procedures.

Program/project manager, field team manager, IDW handling lead, E & E. subcontracting/procurement administrator, and field team members should meet prior to field work to verify that plans and procedures are in place to address IDW handling.

6.2 Waste Minimization

- The generation of IDW should be minimized to reduce the need for special storage or disposal requirements that may result in substantial additional costs yet provide little or no reduction in site risks. proper planning of site activities that may generate IDW;
- using techniques such as replacing solvent-based cleaners with aqueous-based cleaners for decontamination of equipment;
- reusing equipment (where it can be decontaminated);
- limiting traffic between clean and hot zones; and
- using non-intrusive or minimally intrusive investigation methods and sampling techniques that generate little waste (e.g., direct-push samplers).
- Avoid co-mingling of hazardous and non-hazardous wastes.
- Refer to the latest RCRA hazardous waste regulations and the E & E Field Logbook SOP DOC_2.1 for guidance on the types of information to be recorded.

6.3 Non-hazardous wastes

- Decontaminated personal protective equipment (PPE), disposable equipment and clean trash [household-type wastes])
  - for active facilities, request permission from the owner/operator to dispose of non-hazardous wastes (e.g., decontaminated PPE, disposable equipment and clean trash [household-type wastes]) in the facilities' operational trash receptacles/dumpsters.
  - render these wastes unusable (e.g., by cutting or tearing the material).
  - double bag in plastic bags and dispose in dumpster.
  - non-hazardous wastes may also be taken to a permitted landfill for disposal.
- Soil/sediment cuttings, drilling mud, purge or development water, decontamination wash water disposal should be specified in the project planning documents. Unless other applicable or relevant and appropriate requirements or public concerns require off-site disposal, RCRA non-hazardous wastes should be left on site.
  - Soil/sediment cuttings
    - spread around the well
    - place back in the borehole or excavation
  - groundwater (potable water wells, background monitoring wells)
    - pour on the ground in area(s) where no environmental impacts are anticipated
  - groundwater (monitoring wells)
    - pour on the ground in area(s) where no environmental impacts are anticipated
  - aqueous decontamination fluids (may include low levels of non-phosphate detergents)
    - pour on the ground in area(s) where no environmental impacts are anticipated
    - pour down a sanitary sewer if available

6.4 Hazardous wastes

For sites undergoing or scheduled to undergo cleanup/remediation, it may be possible to properly containerize, label, and store hazardous wastes on-site in a designated area of contamination. RCRA regulations impose limitations and restrictions on the transport, storage, and disposal of hazardous wastes. Waste disposal would be handled during the cleanup activities. Program/project planning documents should provide guidance and procedures if this is a viable option for handling hazardous wastes.

- Record keeping for hazardous wastes should be in compliance with applicable or relevant and appropriate requirements.
- Label hazardous waste containers using preprinted labels attached to the side(s) of the containers, complete the labels using indelible ink. Include:
  - Description of waste
  - Contact information
  - Date waste was first accumulated

  - Waste generator number
- For sites with operational treatment/disposal units (TDU), it may be possible to dispose of hazardous wastes in the on-site TDU. Program/project planning documents should provide guidance and procedures if this is a viable option for handling hazardous wastes.
- If off-site disposal of hazardous wastes is anticipated, it should be addressed in the program/project planning documents. Field personnel should conform to the procedures identified in these planning documents.
  - Hazardous wastes will be disposed of at a licensed treatment, storage and disposal facility
  - Treatment/disposal facilities and transporters must pass a compliance verification before being used
- E & E produced hazardous wastes
  - Properly contain and label known or suspected hazardous wastes
  - Use available knowledge of historical site activities and existing site processes/raw materials/waste streams information, site monitoring and/or sample analysis data to identify wastes
  - Program/project planning documents should provide the procedures required for sampling and analysis of wastes if necessary to characterize wastes prior to shipment to a licensed treatment/disposal facility
  - Containerize like wastes (e.g., do not mix organic solvent wastes with aqueous wastes)
  - Verify that labels comply with shipping (e.g., DOT) regulations, receiving treatment/storage disposal facility requirements, and federal, state and local regulations/requirements as specified in the program/project planning documents.
- Subcontractor waste production
  - In cases where a subcontractor (e.g., driller) produces the hazardous waste, the subcontractor should be responsible for proper containerization, labeling, temporary storage, transport, and disposal of the wastes.
  - Subcontracting documentation should include relevant subcontractor SOPs for these activities and specify the documentation to be provided to E & E before, during, and after waste disposal
  - The E & E project manager should verify that subcontractor IDW handling complies with applicable or relevant and appropriate requirements as well as complying with other subcontracting requirements
  - The E & E project manager should verify that the subcontractors proposed transporters and facilities have passed a compliance verification

7 Health and Safety

This SOP does not apply to IDW associated with explosives, radioactive, mixed, or pathogenic wastes. Handling of these wastes should be addressed in program/project planning documents.

Field staff should formally acknowledge that they have read and understood the program/planning documents, especially the site-specific health and safety plan.

If heavy drums need to be moved, appropriate drum-handling equipment should be used. Prior to moving a drum, it should be visually inspected for labeling to verify the contents, signs of deterioration, signs that it is under pressure (bulging or swelling), or leaking. If the drum shows sign(s) of deterioration, leaking, or being under pressure, the drum should not be moved until a proper procedure is determined in conjunction with the project manager and site safety officer.

8 Quality Assurance Quality Control

Keeping hazardous and non-hazardous wastes separate will help control the costs associated with IDW waste handling, transport, storage, and disposal.

The program/project manager should identify personnel for the field team who have knowledge, training and experience in the field activities being conducted. The field team leader or designee is the lead for IDW handling; and will be responsible, with support from other field personnel, for implementing the procedures in this SOP.

The field team leader will verify that disposal information is recorded in the field logbook(s).

The IDW lead and field team manager will verify that shipping of both hazardous and non-hazardous waste complies with DOT and/or other applicable regulations.

9 Special Project Requirements

Site-specific requirements for IDW handling will be found in the program/project planning documents.

10 References

The following list sources of technical information on IDW procedures.

United States Environmental Protection Agency (USEPA). 1992. Guide to Management of Investigation-Derived Wastes (April 1992). 8 pp. OSWER 9345.3-03FS, NTIS: PB92-963353INX. Located online at: http://www.epa.gov/superfund/policy/remedy/pdfs/93-45303fs-s.pdf .

_____. 2010. Operating Procedure, Management of Investigation Derived Waste, US EPA Region 4, SESDPROC-202-R2, October 15, 2010

Scientific, Engineering, Response & Analytical Services Contract (SERAS), Standard Operating Procedures, Investigation-Derived Waste Management, SOP 2049, 10/21/94

United Nations Industrial Development Organization, Cleaner and Sustainable Production Unit, Water Management, POPS Portal, Management of Equipment and Investigation Derived Waste, http://www.unido.org/index.php?id=5584

END OF SOP

ecology and environment, inc.

STANDARD OPERATING PROCEDURE

FISH SAMPLING

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Contents

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1 Scope and Application

This Standard Operating Procedure (SOP) describes the sampling procedures used by E & E field personnel to collect fish for analytical sampling of suspected contaminants. The purpose of fish sample collection is for the determination of the prevalence of contaminants in fish tissue or whole bodies, as well as specific analysis of tumors or other deformities in fish organs. Fish samples for analytical analysis typically consist of whole body collections of specific fish species or specific organs, such as liver samples for monitoring of fish tumors in contaminated waterways. The scope of this SOP is to describe the equipment and procedures used for obtaining representative fish samples, quality assurance measures, proper documentation of sampling activities, and recommendations for personnel safety. Typical applications include the collection of fish samples at contaminated sites, such as a Great Lakes Area of Concern (AOC) or collections from waterways located on or in vicinity of a hazardous waste site. Site-specific sampling procedures vary depending on the data quality objectives (DQOs) identified in program/project planning documents.

Procedures for sample handling are defined in E & E Environmental Sample Handling, Packaging and Shipping SOP ENV 3.16. Site-specific sample handling procedures are dependent on the project DQOs.

Procedures for equipment decontamination are defined in E & E Sampling Equipment Decontamination SOP ENV 3.15. Site-specific equipment decontamination procedures are dependent on the project DQOs.

This fish sampling SOP is intended for use by personnel who have knowledge of biological sampling activities, including the collection of fish specimens or who are working under the direct supervision of someone with such experience. The United States Environmental Protection Agency (USEPA) has developed several manuals which provide additional guidance for these types of sampling events (Barbour et al., 1999, USEPA 2002).

2 Definitions and Acronyms

AOC	Area of Concern
COC	Chain-of-Custody
DNAPL	dense non-aqueous phase liquid
DOT	(United States) Department of Transportation
DQO	Data Quality Objective
E & E	Ecology and Environment, inc.
EPA	United States Environmental Protection Agency
PFD	Personal Flotation Device
SHASP	Site-specific Health and Safety Plan
SOP	Standard Operating Procedure

3 Procedure Summary

Fish sampling procedures will vary depending on the DQOs identified in the planning documents. The primary variations in sampling technique are dependent upon the body of water from which the samples will be collected. For the purposes of this SOP, waterbodies

(aquatic habitats) are limited to those containing freshwater, as all of the more recent E & E projects involving marine waters have utilized subcontractors to perform these sampling activities. E & E fish sampling studies have been conducted on a variety of aquatic habitats including: lakes, reservoirs, creeks, rivers, and wetlands. Procedures and equipment will vary dependent upon which aquatic habitats are being sampled, as well as the DQO for the particular sampling event.

The primary physical variable affecting sampling procedures is the depth of water being sampled; other factors include water velocities, water clarity, and composition of various substrates. The preferred sampling technique is electrofishing either by boat or by using a backpack electrofisher. In certain instances a shore-based system may be used as well, allowing for a larger electricity field to be generated in shallow waters (e.g. wadeable) compared to a backpack electrofisher. Other sampling techniques may involve a variety of different fishing nets including beach seines, gill nets, hoop/fyke nets, or trap nets. In certain instances where game fish are the target of interest, angling may be the preferred sampling technique. Each of these is discussed in additional detail below.

4 Cautions

This SOP is applicable to routine E & E fish sampling and is limited to relatively small collection efforts. Fish sampling for larger projects, where multiple collection sites are involved and large numbers of a variety of fish species are targeted for collection are not routine activities and sample collection procedures should be described in project planning documents.

A large variety of fish species may be collected with the sampling techniques described in this SOP. Field samplers should be familiar with fish behavior and life history characteristics to improve the efficiency of fish capture. In addition, some of the techniques results typically result in mortality of fish (e.g. gill nets) and attempts should be made to limit bycatch of fish species not targeted by the sampling effort.

When sampling with passive gear types (e.g. leaving nets set overnight.), close attention should be made to securing the nets either to the bottom with anchors or to hard structures or trees along the shoreline. The loss of a fishing net would have the potential to cause unwanted bycatch and would be an unacceptable situation to occur.

Depending upon the DQOs, fish may be targeted for collection and retention for analytical analysis (e.g. fish tissue contaminant concentrations). Other studies may only be interested in fish condition and species composition, where all individuals are released alive following processing. Careful consideration should be given to choosing appropriate sampling techniques based on the fish species characteristics targeted for collection, as well as the intended outcome of the sampling effort; released alive on site or retention of fish samples for analytical analysis.

For projects that involve retention of fish for analytical analysis, care should be given to the intended procedures for laboratory analysis. Individual fish may be composited into a larger sample, and individual fish in the composite samples should be of similar size; this will ensure that fish are of similar age, increasing the likelihood that the composite samples from various sampling locations (e.g. contaminated sites compared to reference sites) would have been exposed to the applicable environmental conditions for similar timeframes or exposure times.

To obtain a representative sample of the targeted fish species, E & E personnel should consider the presence of contaminant gradients within a waterbody. This is particularly a concern when targeted fish species are not very mobile and have a smaller home range.

As soon as fish are collected, they should be retained in a live well until processing. Nontarget species collected should be returned to the water as soon as practical. Individuals of the selected target species should be rinsed with ambient water to remove any foreign materials from the external surface, handled using clean nitrile gloves, and retained in a clean live well or cooler with ice prior to processing and ultimate shipment to the laboratory.

In shallow flowing water, where disturbance of the bottom will resuspend sediments, fish sampling should be conducted moving from downstream to upstream. In addition, care should be given to avoid disturbing habitats, in order to maximize the ability to capture fish. In small streams with riffle pool habitats; riffles can be sampled with the nets being secured at the downstream edge. Pool habitats should be sampled by targeting structure or fish holding areas, while minimizing disturbance to unsampled habitats.

Experience has shown that real-world conditions may result in difficulty in capturing targeted fish species. E & E personnel should utilize their experience in capturing the targeted species or sampling representative habitats in orde to adequately estimate required sampling times.

Standard measures, such as the use of disposable gloves, that meet project DQOs, should be used to avoid cross contamination of samples.

5 Equipment and Supplies

The equipment and supplies required for field work depend on the program/project DQOs. The following is a general list of equipment and supplies. A detailed list of equipment and supplies should be prepared based on the project planning documents. In general, the use of dedicated or disposal equipment is preferred but equipment may be re-used after thorough decontamination between sample locations (refer to E & E Sampling Equipment Decontamination SOP ENV 3.15).

- Sampling plan, sample location maps, bathymetric maps, and other applicable spatial data,
- Sampling vessel (for deep water habitats) – includes boat, motor, trailer, oars, gas, personal flotation devices (PFD), and other U.S. Coast Guard safety devices.
- Electrofishing equipment and all associated gear (if proposed for sampling;
- Fish-nets (beach seines, gill nets, fyke-hoop nets, etc.), ropes, anchors, and buoys for marking (if proposed for sampling);
- Angling equipment geared toward the specific aquatic habitat of the targeted species,
- Livewell and/or buckets, and aerators, if needed;
- Measuring boards and weight scales,
- State Scientific Collection permit (also federal permit if federally-listed species are involved),
- Datasheets, logbook, waterproof pens or markers, clipboard,
- Any additional site-specific safety equipment, as specified in the health and safety plan,
- Global Positioning System (GPS) equipment, if needed, as specified in the workplan, and
- Camera.

Supporting equipment and supplies also may be required to address the following:

- Field logbooks and supplies (Refer to project planning documents and the E & E Field Activity Logbooks SOP DOC 2.1 for details)
- Decontamination equipment and supplies (Refer to project planning documents and E & E Sampling Equipment Decontamination SOP ENV 3.15for details)
- Sample containers, preservatives, and shipping equipment and supplies (Refer to project planning documents and the E & E Environmental Sample Handling, Packaging and Shipping SOP ENV 3.16 for details)
- Waste handling supplies (Refer to project planning documents and E & E Handling Investigation-Derived Wastes SOP ENV 3.26 for details)

6 Procedure

6.1 Prior to Field Activity

E & E staff will use the following procedures for completing fish sampling:

- Review relevant project planning documents, e.g., work plan, sampling and analysis plan, quality assurance project plan, health and safety plan, etc.
- Select the sampling procedure(s) that meet project DQOs.
- Refer to the E & E Field Activity Logbooks SOP DOC 2.1 for guidance on the types of information that should be recorded for each sample.
- Refer to the E & E Environmental Sample Handling, Packaging and Shipping SOP ENV 3.16 for guidance on how samples should be labeled, packaged, and shipped.

E & E Project Manager or designated personnel will establish any sampling or laboratories subcontracts.

6.2 Electroshocking

Electrofishing, as the name implies, creates an electric field in water through the introduction of electricity through an anode and a cathode attached to an electrofishing unit and a power source (either a generator or a battery). When a fish enters the electric field, they enter into a taxis state (a muscle response that causes fish to swim toward an anode) and go into narcosis, an immobile state that causes the fish to float belly-up once they are close to the anode. This response only lasts several seconds so biologists need to net the fish quickly prior to them swimming away. At least two biologist are needed to conduct electrofishing, as one is needed to man the equipment, while the other is needed to net and capture the fish.

Electrofishing may be conducted from a boat, a backpack, or a shore-based system. Boat electrofishing is conducted in deeper waters (waters depths greater than 3 to 4 feet); waters that are typically too deep to wade in. Aquatic habitats sampled with boat electrofishers typically include: lakes, reservoirs, or larger rivers. Boat electrofishers utilize two booms off the bow of the boat which act as the anodes and a cathode array or the boat hull for the cathode. As E & E does not own an electrofishing boat, this equipment is typically obtained through a rental or subcontractor agreement, with E & E providing the biologists to conduct the sampling. Technical staff should review operation and safety procedures with subcontractor or vendor prior to use.

Backpack electrofishers are typically used in smaller streams or shallow water areas (e.g. wetlands) that are easily wadeable. E & E owns a Smith Root battery-powered backpack electrofisher or may be rented depending upon location of the project. Biologists should thoroughly read the Owners Manual and adhere to manufacturers specifications and safety procedures prior to use.

Actual sampling techniques will be dependent upon results of the fish tissue data collection effort and the effectiveness of various techniques and suitability of habitat. If unforeseen circumstances arise that impact this scope of work, E & E field sampling should determine an appropriate course of action and the associated cost implications with the project manager.

The time for each electroshocking run should be established in the project planning documents. Sampling collection efforts for each electroshocking run are typically about 15 minutes. The allotted time amount may changed based on project DQOs to allow sample results to be comparable to historical sampling events. A similar level of effort should be implemented at each site to ensure data comparability. The expected number and length of the sample reach should be established for each site. Overall reach lengths will be determined in the field, but lengths typically fall within the range of 500 – 1,000 feet. GPS coordinates of the start and stop locations for each site can be collected to provide appropriate documentation so future monitoring efforts can sample the same locations.

At completion of electroshocking run, fish immobilized during each run will be dip-netted and put into aerated live wells for processing. Fish are processed as described in Section 6.4.

6.3 Fishing Nets

Fishing nets may be used in aquatic habitats that are not conducive to electrofishing. These areas include deeper water areas (e.g. 6 feet or greater) or in shallow clear waters that are devoid of structure. Beach seines are a common technique to sample shoreline or shallow water areas. Beach seines are most suitable in areas with a relatively smooth bottom, as the seine is dragged along the bottom to corral fish into a confined area for collection. Areas with lots of large woody debris or large rocks on the bottom will tend to foul the beach seine set and not allow the effective capture of fish. Gillnets may be deployed in deeper waters areas, or set overnight to catch nocturnal game fish species; typically, gillnets are used to catch adult fish and can be set on the bottom, in mid-water areas or on the surface. Hoop nets or fyke nets contain a series of hoops or fykes which collect fish by entrapment; they are set in slow water areas where panels can be attached to the sides to try to capture fish from a larger area by corralling them into the hoops or fykes.

Fish collected by netting shall be removed from net cod ends, and placed in an aerated live well for processing. Net set time and run times shall be recorded for each net location, in order to calculate catch per unit effort. Additionally ancillary information that shall be recorded for net samples shall include water depth, in-stream habitat characteristics, and relative water flow characteristics.

6.4 Fishing Processing

Collected fish from each location will be placed into a live wells filled with ambient water and each individual fish will be examined by a qualified biologist and identified to the species level. Total length to the nearest millimeter and weight to the nearest gram will be recorded for each fish. If positive identification cannot be made in the field, specimens will be placed in 10% formaldehyde and stored in labeled in zipper closed bags for identification using standard keys. Identification of specimens will be to the lowest practicable taxonomic level using a taxonomic

key relative to the location. For example, in New York use one or more of the following taxonomic keys:

- Smith, L. 1985. The Inland Fishes of New York State. The New York State Department of Environmental Conservation. Albany, NY.
- Kraft, C. E., D. M. Carlson, and M. Carlson. 2006. Inland Fishes of New York (Online), Version 4.0. Department of Natural Resources, Cornell University, and the New York State Department of Environmental Conservation.

The project planning documents should indicate if voucher species are required. A voucher specimen is archived to serves as physical evidence of the any identifications and descriptions. For routine fish sampling, voucher specimens are not needed to document quality assurance of field identification, except in the case where the field biologists are unfamiliar with a specimen. With the exception of these unknown specimens, all collected fish not retained for bioaccumulation assessment will be returned alive to the site at which they were collected.

External lesions, anomalies, and parasites will also be cataloged and recorded by the biologists for each fish. Photographs will be taken of all fish exhibiting tumors, lesions, or other deformities, with the appropriate labeling (sample collection date, location, species, etc.) shown next to the fish for photo-documentation.

Batch processing of samples may be performed if the number of specimens of the same species in a given sample exceeds 50 or a number identified in the project planning documents. In such circumstances, 50 specimens of a given species shall be processed as stated above (individual length and weight measurements), thereafter the species and size category count will be estimated by sub-sampling. A sub-sample of 30 individuals will be weighed and the total sample will be weighed. The number of individuals in the whole sample will be estimated from the ratio of the total sample weight to the sub-sample weight total and the count within the sub-sample.

If fish are to be analyzed for contaminates in the tissue, then all fish will measured and weighed as noted above and the appropriate specimans selected for analysis. Then specimens will be placed in food grade plastic bags. The specimens will be double bagged with a waterproof individual label and placed on ice. E & E recommends that whole fish be processed at the laboratory for analysis by excising standard filets (scales removed) and reserving the remaining carcass. If necessary the processing can be performed in the field, but more accurate scales may be required that for weighing whole fish. Addiitonal care is required to prevent cross contamination of the tissue.

The weight of the standard filets and the carcass will be individually weighed and the individual weights recorded as the carcass weight. Total weight of the samples following preparation will be recorded in the laboratory. Some sample mass loss (e.g., liquids) is expected during the preparation of samples but efforts will be made to retain as much of the original sample material as possible. Each sample will be stored frozen until ready for analysis. Samples will be transported under chain of custody to the laboratory.

If the fillet sample collection procedure (based on NYSDEC Fish Preparation Procedures for Contaminant Analysis; NYSERDA 2008) to be implemented at the laboratory:

- Use dedicated equipment (scalpel, fillet knife, forceps) for this procedure;
- Remove scales from the fish. Do not remove the skin;

- Make a cut along the ventral midline of the fish from the vent to the base of the jaw;
- Make a diagonal cut from base of cranium following just behind gill to the ventral side just behind pectoral fin;.
- Remove the flesh and ribcage from one-half of the fish by cutting from the cranium along the spine and dorsal rays to the caudal fin. The ribs should remain on the fillet;
- Score the skin and weigh the fillet and carcass;
- Homogenize the entire fillet;
- Place fillet in a labeled glass jar; and
- For fish less than 6 inches, fish are prepared by cutting the head off from behind the pectoral fin and eviscerating the fish. Ensure that the belly flap is retained on the carcass to be analyzed. Report this modification in the field notes or laboratory report.

6.5 Field Data Collection

Field data collection activities will be recorded using project specific field data sheets; similar information will be collected in the field during the targeted fish collection activities. For each sampling event, the following field data will be recorded:

- General Sample Identification
  - Date
  - Start time, Stop time
  - Weather, field team members
  - Sample collection method
  - Sample location
  - Sample identification
  - Sample collectors
- Water Quality (if required)
  - Water body
  - Temperature
  - Dissolved oxygen
  - pH
  - Conductivity
- Biological Data
  - Species common name
  - Length
  - Weight
  - Status/condition (live, dead, moribund)

- DELT (disease, erosion, lesions, tumors – see additional datasheet for gross body observations for brown bullhead)
- Batched fish (length category, count, and weight)

6.5.1 Water Quality

In-situ water quality data, including pH, dissolved oxygen, and conductivity are often measured at each fish sampling location during each event. Water quality measurements are recorded on field data sheets. Water quality instruments are subject to calibration prior to and following each field sampling event. Additionally, water quality instruments shall be properly maintained while in the field to ensure their accuracy. See Surface Water Sampling SOP 3.12 for additional information if water quality monitoring is required.

7 Quality Assurance/Quality Control

Program/project planning documents provide project-specific procedures to be used in compliance with quality assurance and quality control measures. Fish sampling designs will typically specify the target specifies and number of samples to be statistically valid. The sampling design may also specific length and weight of fish to meet specific design objectives. All field identification of fish should be conducted by qualified personnel. If targeted species and catch per unit effort do not meet the sampling design, then alternative species may be necessary. Adjustment to sample locations, season or other factors may need to be adjusted. Field decisions on sampling deviations need to be approved by the project manager.

8 Health and Safety

Health and safety procedures should always be thoroughly considered during fish sampling events as there are a number of hazards that need to be avoided by field personnel. Working in water can create exposure issues, as well as accidental drowning. In addition, dependent upon fish sampling gear, field samplers can be exposed to other hazardous conditions (e.g. electricity). Boats and electrofishing gear should only be operated by qualified personnel, with experience in their proper use. Whenever fish sampling is occurring from boats, each field team member should where PFDs. When electrofishing, each team tember should where rubber boots and gloves. During deployment and retrieval of fish nets, care should be given to securing or retrieving ropes and anchors to avoid entanglement in gear.

9 Special Project Requirements

Project or program-specific requirements that modify this procedure should be entered in this section and included with the project planning documents.

10 References

The following list sources of technical information on fish sampling.

Barbour, M. T., J. Gerritsen, B. D. Snyder, and J.B. Stribling. 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates, and Fish. Second Edition. EPA 841-B-99-002. U.S. Environmental Protection Agency; Office of Water, Washington, D.C.

U. S. Environmental Protection Agency (USEPA). 2002. Field Sampling Plan for the National Study of Chemical Residues in Lake Fish Tissue. Office of Water, Washington, DC. EPA-823-R-02-004

END OF SOP

B Laboratory Procedures

AQUATEC BIOLOGICAL SCIENCES, INC.

QUALITY ASSURANCE PROGRAM PLAN

March 2012
Revision 13

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1.0 QUALITY ASSURANCE POLICY STATEMENT

Policy

Aquatec Biological Sciences, Inc. (Aquatec) shall provide testing services that:

- Conform to our clients needs, requirements, and intended use,
- Comply with applicable government standards and regulations,
- Comply with current laboratory accreditation standards, including the State of New Hampshire Department of Environmental Services Environmental Laboratory Accreditation Program (NH ELAP) as defined in the National Environmental Laboratory Accreditation Conference (NELAC) standards. When The NELAC Institute (TNI) standards are implemented, incorporate the new standards into Aquatec's Quality System.
- Laboratory personnel must remain impartial and free of commercial, financial, or other pressures which might influence their technical judgment.

Continuous efforts toward improvement are integral to laboratory activities. These improvements ensure that Aquatec maintains the high standards defined by NELAC and other regulatory performance requirements.

Purpose

The purpose of this manual is to establish the framework and to assign responsibilities to ensure that all employees conform to the policy. Additionally, this manual is used to inform appropriate regulatory authorities and clients of quality practices at Aquatec. This Quality Assurance Program Plan (QAPP) formalizes the Quality System that has been established and that is in operation for all sample-related activities including employee training, sample handling, sample preparation, sample analysis, and documentation. Aquatec's Employee Manual and Chemical Hygiene Plan supplement the QAPP as components of our Quality System.

Scope

This QAPP applies to all employees. Aquatec requires a firm commitment from all members of this laboratory to follow a comprehensive Quality Assurance Program Plan. This commitment and dedication to quality is fully supported from the bench level to

upper management in order to meet the objectives of our laboratory and best serve our clients.

This QAPP undergoes an annual review by the Director and/or Department Managers. Any revisions to the QAPP are distributed throughout the laboratory to replace previous editions so that only the most current version is available (prior editions are stored in Administrative files). It is the responsibility of the Director to ensure that all employees familiarize themselves with and comply with the procedures presented in the most current manual and any associated documentation.

The policies and practices of quality assurance/quality control presented in the following text are set forth as minimums. Reading of the most current version of the QAPP and the companion document, the Employee Manual, is required. Formal signature page documentation of reading and understanding of the current edition of the QAPP is required.

2.0 FACILITY DESCRIPTION AND SECURITY

2.1 Laboratory Facilities

Aquatec has been active in environmental investigations and sample analysis since it's founding in 1996. Aquatec is located at 273 Commerce Street, Williston, VT.

The floor plan in Appendix E (Figure 1) shows the location, size, and utilities available. Aquatec has the support systems for biological testing and analysis including: refrigerated sample storage capacity, freezers, reverse osmosis and organic removal (carbon) and de-ionizing water purification systems, computer networking, controlled-temperature laboratories and systems, water quality monitoring equipment, microscopes, drying oven, incubators, boats, field sampling equipment, and other equipment associated with microbiological, toxicological, and ecological tests or investigations.

Aquatec's laboratory design accommodates segregated sample receiving and log-in activities. Potentially incompatible activities can be segregated by using combinations of laboratory space. A ventilation hood located in the Limnology Laboratory isolates volatile organic compounds, acids, or other potentially noxious materials from other laboratory spaces. Microbiology analyses are performed in the Microbiology/Limnology laboratory. In-house organism cultures used for toxicity testing are separated from toxicity testing activities by covering them with glass or plastic covers or holding them in

incubators. Organisms being acclimated or held for toxicity testing are maintained in glass aquaria, glass bowls, or covered Tupperware-type containers.

2.2 Security

2.2.1 Laboratory Security

Employees are instructed in Aquatec's security policies (e.g., sample security, door policies, data filing, etc.). Because of the nature of our work, security of the facilities, equipment, and project files is necessary. Access to the laboratories on a daily basis is limited to employees. Visitors, regulatory personnel, or clients are greeted at the entrance. If access to laboratory spaces by the visitor is warranted, an employee will accompany the visitor into those spaces until the time that the visitor departs Aquatec. Access doors to Aquatec are maintained in a locked mode during non-business hours.

2.2.2 Data Security and Client Confidentiality

Aquatec employees are expected to be familiar with and adhere to standards of confidentiality mandated by individual contracts and common sense business practices. Confidentiality procedures may include signing project-specific confidentiality agreements and obtaining client permission to release information in other forums (this may exclude those data required to be released through court orders or other legally-binding measures). Administrative actions for possible breaches in confidentiality are outlined in the Employee Manual (Section 1.8).

2.2.3 Computer Security

Access to specific PC's at Aquatec is protected by password-protection. Sensitive data and data files are stored on our Dell server. These files have various levels of security protection dependent upon the nature of information. Files that are routinely available for use by authorized personnel have the broadest level of security- read/write access. Files that are necessary for communication of information are more restricted-access for read only provided to those authorized in the workgroup. The most restrictive files do not have share access and aren't available to be listed by non-authorized individuals.

The file sharing security is dictated and managed by the department manager when routine and communication files are needed. The restricted access files are dictated and managed by the Director. The server is password protected and therefore unauthorized access directly through physically logging onto the server is minimized.

Administrative actions for possible breaches in confidentiality are outlined in the Employee Manual (Section 1.8).

3.0 ADMINISTRATIVE ORGANIZATION

3.1 Personnel Qualifications and Records

3.1.1 Minimum Requirements

Resumes of our key staff, experience, and educational profiles can be found in Appendix A.

3.1.1.1 Director / Quality Assurance Officer Qualifications

The Laboratory Director may also be the acting Quality Assurance Officer (QAO). The Laboratory Director / Quality Assurance Officer must hold a M.S. or higher degree in Biological Sciences and have a minimum of five years experience in environmental biology (three years of academic training past the M.S. level can be applied to the experience requirement). The Director should also have leadership ability, communications skills, and the ability to maintain and improve laboratory operations. As QAO the Director must be knowledgeable in the most recent Quality Systems Accreditation Standard published by NELAC and hold overall responsibility for Aquatec's Quality System. The Director may appoint a Deputy QAO who may assist and also be responsible for some aspects of the Quality System.

3.1.1.2 Department Manager Qualifications

Department Managers must, as a minimum, hold a Bachelor's Degree in a relevant scientific field of study (e.g. biology, microbiology, ecology, environmental biology, and environmental chemistry). In addition, Department Managers must have at least five years of experience in the area of responsibility and be knowledgeable regarding applicable regulatory requirements and published protocols. Department Managers should also have leadership ability, communications skills, and the ability to maintain and improve laboratory operations.

3.1.1.3 Laboratory Analyst Qualifications

A Laboratory Analyst is defined as an employee responsible for generating or recording scientific data from on-going tests or investigations. Full-time Laboratory Analysts have, as a minimum, a Bachelor's Degree in a relevant scientific field of study. Laboratory

Analysts must complete the training requirements outlined in Section 4.3. Requirements for performing specific laboratory skills (e.g., washing glassware, using a balance, colony counting, instrument use, or working on toxicity tests) are outlined in the pertinent SOPs for the specific tasks. Documentation of reading, demonstration of understanding, and agreement to follow the procedures outlined in SOPs and the most recent version of the QAPP, Employee Manual, and Chemical Hygiene Plan is incorporated into our training program for Laboratory Analysts.

3.1.1.4 Laboratory Technician Qualifications

Part-time or temporary Laboratory Technicians must have completed High School and have one year experience in the laboratory section where they are working or had one or more college courses in the biological sciences with emphasis on a relevant field of study.

3.1.2 Personnel Records

Records of employee education, technical background, and training are maintained in Administration Files. Upon hiring, files will be started for the new employee and remain confidential with access by administrative personnel. Outside audits will be permitted to view only the technical information as needed for audit purposes unless specific permission is given by the Director. Some of the documentation associated with initial and continuing training may be filed within SOP notebooks, laboratory training files, or general QA files for the current year. The initial filing of documents will include the following:

Initial Documentation:

Technical (Available to the Director and Department Managers)

1. Resume and any training certificates earned
2. Signed acceptance of the QAPP
3. Signed acceptance of the Chemical Hygiene Plan
4. Signed acceptance of the Employee Manual
5. Signed acceptance of the ethical and legal responsibilities and potential punishment (as included in the Employee Manual and Data Integrity Training)

Administrative (Available to the Director and Administrative Assistant)

1. Copy of employment offer
2. Payroll Information
3. Health Enrollment Forms

Ongoing Documentation may include:

Technical (Available to the Director and Department Managers)

1. Updates of resumes
2. Training Certificates and/or other training documentation
3. Signed acceptance of current QAPP, SOPs, etc.
4. Annual QA proficiency demonstrations if applicable
5. Performance reviews

Administrative (Available to the Director and Administrative Assistant)

1. Personnel Review
2. Updates of payroll and health information
3. Letter of termination and exit interview

3.2 Roles and Responsibilities

In accordance with NELAC requirements, all Aquatec personnel must remain impartial and free of any undue commercial, financial, or other pressures which might influence technical judgment or bias analytical results, data, or reports submitted to clients. It is expected that employees will generate data in compliance with this QAPP and the Employee Manual.

3.2.1 Director / Quality Assurance Officer (QAO) Responsibilities

The Director is ultimately responsible for ensuring data quality and providing technical direction at Aquatec. The Director/QAO develops policies and quality assurance strategies in collaboration with Department Managers and he, along with Department Managers, is responsible for initiating actions to prevent or minimize departures from the Quality Systems program. Additionally, the Director is responsible for reviewing all new non-routine incoming work and making sure that the appropriate facilities and resources are available before commencing such work. The Director is also responsible for general management of the laboratory, scheduling and execution of

testing, and release of testing data and results. Some of these responsibilities may be delegated to Department Managers. Any discrepancies in methodology, procedures, QC criteria, or reporting will be communicated to the Director. When Managers detect discrepancies or problems, they are reported to the Director/QAO who in conjunction with Managers is responsible for developing a corrective action procedure. The Director will also review and provide technical and statistical insight and acceptance/rejection criteria for scientific experimentation that does not have regulatory protocol-driven criteria.

While the Director/QAO is responsible for the preparation and maintenance of the QAPP, updates and revisions may be suggested and completed by Laboratory Managers or analysts, with final approval by the Director. The Director may assist Department Managers or analysts in the preparation, revision, and distribution of SOPs. The Director/QAO must have general knowledge regarding the analytical procedures and methods performed in the laboratory and for which data review is required. The Director/QAO acts as the official laboratory contact for audits, performance evaluation studies and project-specific quality control issues. The Director/QAO approves and confirms the implementation of documented corrective actions for events of non-compliance and also may perform internal audits. The Director may appoint a Deputy QAO as responsible for performing some of the Quality Systems functions.

The Director/QAO is responsible for developing acceptance / rejection criteria for procedures where no method or regulatory criteria or published guidance exist. In cases where no method or regulatory criteria or published guidance are available, the Laboratory Director or designee will communicate with the client to determine the overall objectives of the study. He will also use his best professional judgment in combination with the defined scope of work to develop acceptance / rejection criteria *a priori* to initiating the study. In certain experimental investigations it may not be possible to define exact "control" acceptability criteria, however as a minimum, the experimental environmental conditions should be within a range supporting any testing conducted.

The Director will also ensure that all staff members are made aware of their respective designated responsibilities and that they are fully aware of the extent and limitations of their responsibilities.

The Director will afford clients (or their approved representatives) opportunities to clarify their requests, work plans, or contracts and will monitor laboratory performance in relation to the work performed. Clients occasionally request a site visit and these requests are typically scheduled at the earliest mutual convenience.

The Director is responsible to ensure that the following NELAC-required activities are completed on at least an annual basis:

- Review policies and procedures leading to contractual or other mutual agreements between Aquatec and clients
- Conduct an internal audit of laboratory operations;
- Update the Aquatec Biological Sciences, Inc. website (if active) to reflect current accreditation status;
- Provide managerial oversight and conduct a Management Review to assess the effectiveness of our Quality Systems Program (See Figure 10, Management Review Checklist);
- Conduct data integrity training

3.2.2 Department Manager Responsibilities

Department Managers are responsible for the training and daily operations of laboratory staff. Training of laboratory personnel includes documented reading of the QAPP, Employee Manual, and documented reading of relevant SOPs. Laboratory personnel are expected to have a working knowledge of the Quality Assurance Program Plan (QAPP). Copies of the most current edition of the QAPP and SOPs are made available to each employee for reading and for signature.

Department Managers must have a general knowledge of the test methods within his/her purview for which data review is required and Department Managers are responsible for the flow of work and data produced in their laboratories. Department Managers report directly to the Director. Each Department Manager has analysts who report directly to them. Department Managers are responsible for the maintenance of SOPs and the distribution and enforcement of the QAPP and SOPs in their laboratory sections. Discrepancies in Quality Control (QC) criteria will be brought to Department Manager attention, and a decision will be reached as to whether or not the data are acceptable, typically in consultation with the Director. If, in the judgment of the Department Manager, there are technical reasons which warrant the acceptance or rejection of what appears to be out-of-control data, these reasons must be documented and discussed with the Director before the sample data are reported or plans are made for re-sampling or re-analysis.

Laboratory Managers assign laboratory analysts for lab operations and project-specific tasks at hand. Written and verbal information is provided to the responsible employees so that continuity of service and understanding of the clients' needs and protocol

requirements are fulfilled. Laboratory Managers also communicate with clients to ensure that the scheduling, workflow, and data quality meet the project objectives and provide data review and report preparation. Laboratory Managers also assist on development of work plans and preparation of proposals for new work.

Laboratory Managers, in consultation with the Director, may be responsible for developing acceptance / rejection criteria for procedures where no method or regulatory criteria or published guidance exist. In cases where no method or regulatory criteria or published guidance are available, the Manager will communicate with the client to determine the overall objectives of the study. He will also use his best professional judgment in combination with the defined scope of work to develop acceptance / rejection criteria *a priori* to initiating the study. In certain experimental investigations it may not be possible to define exact "control" acceptability criteria, however as a minimum, the experimental environmental conditions should be within a range supporting any testing conducted.

With the approval of the Director, Managers may also afford clients (or their approved representatives) opportunities to clarify their requests or work plans and will monitor laboratory performance in relation to the work performed. The Director and Managers will review proposed or current projects and insure that proper methods, capabilities, and facilities are available to meet client and project requirements.

3.2.3 Laboratory Analyst Responsibilities

Laboratory analysts are responsible for the generation of data by analyzing samples according to written SOPs or project protocols. Analysts report to their respective Department Manager. They are also responsible for ensuring that documentation related to specific samples is recorded in real time and is complete and accurate. Analysts must provide their Department Manager with immediate notification of problems within the laboratory that could potentially affect the quality of the data. While Managers and the Director have the final authority to accept or reject data (based on compliance with well-defined QC acceptance criteria), laboratory analysts often conduct a preliminary review of data to assess acceptability of the data. Acceptance of data, which falls outside of QC criteria or is questionable in nature, must be reviewed and approved by the Department Manager or the Director. Analysts also provide support in organism culture and handling, water and media preparations, glassware cleaning, and many other tasks associated with microbiological, toxicological, or ecological tests and investigations.

3.2.4 Laboratory Technician Responsibilities

Part time and temporary analysts may perform certain components of laboratory operations under the direct supervision of laboratory manager or qualified laboratory analysts.

3.3 Employee Training

The QAPP, The Employee Manual, and our comprehensive Standard Operating Procedure (SOP) program provide the foundation for training new or current employees. The QAPP and the Employee Manual are required documented reading for new employees. The new employee must also sign an "agree-to follow" statement as part of the documentation record. As the employee gains experience and begins to become responsible for implementation of specific methodologies or protocols, the associated SOP must be read and understood and documented before an analyst becomes qualified for that procedure. The Laboratory Manager or a qualified analyst will also provide a verbal description and demonstrate the procedure to the trainee.

Records of initial training for analyses under the National Environmental Laboratory Accreditation Program (NELAP) will be recorded on the "Demonstration of Capability" form (Appendix E). This form (or a similar form providing the required information), along with supporting documentation should be stored in the personnel files for the trainee analyst. Some aspects of training records may be stored in other readily accessible locations (e.g. SOP signatures are located in the SOP Log).

3.3.1 Orientation

Personnel training procedures begin with an orientation program designed to familiarize the new associate with safety and chemical hygiene issues, the importance of quality assurance/quality control in the analytical laboratory, and company policies and benefits. New personnel are also educated in the ethical and legal responsibilities of the work and recording or reporting data, as outlined in the Employee Manual. This includes potential punishments or penalties for improper, unethical, or illegal actions (Employee Manual Section 1.8). The new employee must also sign an "agree-to follow" statement as part of the documentation record for the Employee Manual and the most current QAPP.

The level of training necessary to perform analytical tasks is derived from academic background, past experience, technical courses, and on the job training for specific

methods or instrumentation. The responsibility for formal academic training lies foremost with the individual, and educational background checks are fundamental to hiring. The responsibility for the additional specialized skills obtained through in house training or external workshops is an obligation shared by the individual and their supervisor based upon expertise and experience needed in the laboratory.

An individual's academic and professional experience is kept on file including an initial statement of qualifications or resume and any additional documentation concerning prior experience or subsequent training. Copies of certificates of completion, transcripts, diplomas, or other documentation will be included in the files as appropriate.

An employee's training must include documentation that the employee has read, understands, and agrees to follow the latest version of our Quality Systems documentation (QAPP and the Employee Manual). Training on specific instrumentation or laboratory procedures is SOP-driven, with signature documentation of reading and understanding and "agreement to follow" for SOPs. Documentation of training specific training activities must be included with the employee's training records. Documentation of reading current SOPs is stored in the SOP Log. Training records are available for inspection by appropriate regulatory or certifying authorities.

Trainees are under the supervision of experienced analysts who are responsible for showing them the analytical procedures including applicable QA/QC measures. A new analyst will not be permitted to fully implement an analysis until their supervisor is confident that the analytical and QA/QC procedures can be carried out correctly and method proficiency is documented (Demonstration of Capability Appendix E).

3.3.2 Data Integrity Training

Data integrity is the foundation of our work at Aquatec Biological Sciences, Inc. Our initial and annual training program includes discussion of the importance of data integrity and how we achieve this objective. Key elements of our data integrity training include:

- Discussion of Aquatec's organizational mission
- Record observations in real time;
- Correct recording errors in a manner that doesn't obliterate original records;
- Prevent improper data manipulations;

- Maintain confidentiality regarding the specifics of our work (unless authorized to release information by our clients);
- Maintain an environment of honesty and full disclosure in analytical reporting;
- Communicate to employees that breach of ethical behavior could result in investigative actions, including possible termination or prosecution.
- Employees must remain impartial in their judgment and free from any commercial, financial, or other pressures that might influence their technical judgment.
- Data Integrity Training will be provided as a formal part of new employee training and on an annual basis for current employees.

Signed documentation of data integrity training is required. Any issues related to data integrity may be discussed privately with the Director. Data integrity procedures and elements of training will be reviewed by the Director annually and updated or modified as deemed appropriate.

3.3.3 Initial Training for Analysts

A "method" refers to a NELAC-accredited method or specific instrument used in support of a NELAC-accredited method. Aquatec is currently accredited for whole effluent toxicity (WET) testing for several US EPA WET testing methods (Appendix D). These methods involve a complex sub-set of laboratory skills and performance through several days. The duration of WET tests require that several analysts may be involved in different components of a method during any one day or over several days. Initial training should incorporate all of the sub-tasks associated with the method. The initial training will introduce the new employee to the aspects of a particular method over time through explanation and demonstration of the procedures. Training for individual components of each method must be documented and must include reading and understanding of the method SOP. This training can be accomplished by a new employee through supervised participation in reference toxicant tests or client-based tests. The ability to record observations and measurements that are consistent with those by trained employees is a key component of required training.

When, in the judgment of the trainer, the new employee has demonstrated proficiency in the overall method, the trainer must complete a Demonstration of Capability (DOC) for that method for that individual. A DOC is required for each NELAC-accredited method for each employee before that individual can participate in all aspects of the method without supervision. Supporting documentation of training-elements must be attached

to the DOC. The DOC must be signed by the Department Manager and the QAO (or Deputy QAO) to certify that training for that method has been completed.

3.3.4 Documentation of On-Going Proficiency

Aquatec analysts must remain proficient in the tests that they perform. Quality Systems documents and SOPs are reviewed and revised (if needed) as part of the annual Quality Systems review and analysts must read and agree to follow the latest versions of these documents. SOPs are revised periodically to reflect advances or changes in regulatory-based protocols or editorial corrections.

Analysts will be considered up to date if their training for NELAC-accredited methods contains documentation that they have read, understood, and agreed to perform the most recent version of the test method (as outlined in current SOPs). In addition, documentation of continued proficiency for NELAC-accredited methods must be completed at least annually by one of the following. Since WET methods require several days to complete and several analysts, observations (e.g. organism counts) consistent with prior or subsequent observations (by other analysts) will fulfill this requirement.

On-going proficiency can be assessed through:

- Documented ability to participate in client-based tests that meet negative control acceptance criteria.
- Documented ability to participate in DMR QA tests that result in graded acceptable performance.
- Documented ability to participate in positive control tests (reference toxicant tests) that meet performance criteria and control chart criteria.

Records of training updates and demonstrations of proficiency must be maintained.

3.3.5 Work Cells

Work Cells are defined as a group of analysts working on parts or cooperatively on all of a laboratory method and generating data for that method (e.g. as noted above, a toxicity test may require different analysts to perform different tasks as part of the overall method or may perform the same tasks for a method but on different times or days). Analysts comprising Work Cells must, as a unit, meet the requirements outlined in the Section above. Each member of a work cell must meet the requirements of initial

training and on-going proficiency, such that the individual can participate in any aspect of work assigned to the work cell. However, some participants in a work cell may be qualified for limited portions of the overall assignment. These individuals must have documented training for their specific assignments.

3.3.6 Safety Training

Aquatec has a fundamental responsibility to provide facilities, equipment, maintenance, and an organized program to make necessary improvements to ensure a safe working environment. Unless employees fulfill their responsibilities for laboratory safety, the safety related features of the facility and established safety programs will be ineffective.

The laboratory is equipped with many structural safety features. Each associate must be familiar with the location, use, and capabilities of general and specialized safety features associated with their workplace. To protect employees from potential workplace hazards, Aquatec provides and requires the use of certain items of protective equipment. These may include safety glasses, protective clothing, gloves, respirators, etc., as needed for the tasks at hand. Repeated violations of published safety practices (Employee Manual, Section 4.3) may result in punitive action ranging from required safety training to employment termination.

3.4 Laboratory-wide training for new or modified methods

If Aquatec applies for accreditation within a new field of NELAC accreditation, laboratory-wide expertise in the proposed field of accreditation must be demonstrated through completion of a laboratory-wide Demonstration of Capability (LW DOC). This DOC must be completed before accepting real-world samples for the method. Work cells, as a group, must participate in the LW DOC. For new fields of accreditation involving WET testing, a series of five standard reference toxicant (SRT) tests must be completed and an SOP must be written in NELAC format for the specific method, approved, and read by analysts. The controls for these SRT tests must meet acceptance criteria and the endpoint values (e.g. LC50 or IC25) plotted on a control chart to establish limits or sensitivity to the selected toxicant for that species.

3.5 Review of Requests, Tenders, and Contracts

Accreditation under the NELAC Standard requires that Aquatec provide a policy and procedures for review of requests for laboratory services and contracts. The Director of Aquatec Biological Sciences, Inc. is responsible for this review, however, Department

Managers may also review new projects specific to their area of expertise. Most requests for services are received as informal telephone requests or e-mail communications, while some projects require formal contractual proposals and signatures. In either case, the elements of this review must include:

1) **Project-specific requirements, including the analytical methods must be clearly defined, documented, and understood.**
    a. Routine methods: Typically, methods for new or continuing projects are routinely and very frequently performed at Aquatec (e.g. whole effluent toxicity (WET) testing, whole sediment tests). For projects requiring routine methods, a formal review of capabilities is not required because physical resources are available and personnel are qualified to perform the analysis. In communications with clients, either the Director or Project Manager makes decisions regarding the acceptance and scheduling of projects requiring routine analyses. Once a project is accepted, project-specific documents (e.g., permit, costing information, client communications, etc.) should be assembled in a unique project folder prior to the initiation of the project (Project File) and a project-specific electronic folder should also be established for project documents. NELAC accreditation for WET methods and method-specific Standard Operating Procedures (SOPs), and standard reference toxicant control charts define and document our experience with analyses routinely conducted at Aquatec. Formal contracts for projects requiring routine analyses must be reviewed, signed by the Director (or his designate), and returned to the client as a signed document. A copy of the signed contract must be stored in the project folder.
    b. Non-routine methods: Occasionally, clients request test methods that are not routinely performed at Aquatec. In this case, the Director, Department Manager, or Project Manager must assemble the applicable documents describing the specific method(s) (if they are available) before the project is initiated. The proposed methods must be evaluated by the Director or Department Manager to determine whether Aquatec has the resources and expertise to perform the requested analyses. If a decision is made not to proceed with requested work, the justification for this should be documented (e.g., in telephone logs or e-mail communications) and related to the client. If a decision is made to proceed with the requested work, copies of communications between the client and Aquatec that reflect any

modifications of methods or final descriptions of the methods, or other communications should be stored in the project file. Formal contracts for projects requiring non-routine analyses must be reviewed, signed by the Director, and returned to the client as a signed document. A copy of the signed contract must be stored in the project folder.

2) **The laboratory must have the capability and resources to meet the requirements of the contract.**
   a. Contracts or Agreements Requiring Routine Methods: The elements of capability are described in Section 1) a. immediately above. By virtue of our NELAC accreditation, SOPs, PT performance (e.g., annual DMR/QA Study), and on-going training, Aquatec maintains a continuous capability to perform frequently requested analyses. The primary consideration in reviewing requests for services is scheduling. The Director or the Department Manager is responsible for determining the timing of new work, so that the work can be performed at a time when adequate qualified personnel and laboratory space are available. The Department Manager may prepare a calendar illustrating current and future projects to ensure that sufficient physical resources and personnel are available to accomplish the requested work in a time-frame that will meet the client's needs. Aquatec must inform the client if any potential conflicts, deficiencies, or lack of appropriate accreditation status (if applicable) could in any way affect our ability to accomplish the requested work.
   b. Contracts or Agreements Requiring Non-Routine Methods: For those projects requiring non-routine or non-NELAC fields of accreditation, the Director or Department Manager must assemble documents describing proposed methods and any additional documents describing the overall objectives and requirements of the proposed project. This may include, but not be limited to: Project-specific Quality Assurance or Sampling and Analysis Plans, project-specific SOPs, documents acquired from relevant peer-reviewed publications, and documents describing the overall Scope of Work. The Director or Department Manager, must review these documents to determine whether Aquatec has the capabilities, expertise, and resources to perform the requested work. Once an understanding of the scope of the requested work is attained and an agreement is reached with the client, documents generated during this developmental process must be filed in the project file. The results of any preliminary tests (e.g. performed to

learn a new procedure) must also be stored in the project file or other readily accessible location. Also, the Department Manager must be directly involved in the development of new procedures, including initial experimentation, preparation of SOPs for the procedure(s), and initial training of laboratory personnel.

3) **The review must ensure that the appropriate test method has been selected and the client's objectives will be met through performance and completion of the requested methods.**
    a. In most cases, it is the client's responsibility to request the appropriate method(s), whether for compliance testing/monitoring or for more wide-scale ecological assessments because the client is likely to be operating within a regulatory framework. Typically the methods are permit-driven or regulatory agency-driven and modifications or method substitutions are not allowed.
    b. If we suspect that an inappropriate analysis has been selected (e.g. using freshwater species for salt water exposures) we will suggest a modification of the method or test protocol to assist the client in making the appropriate choices or we will suggest that he/she contact the appropriate regulatory agency (if applicable) for clarification. Records of modifications for work should be documented in telephone logs, e-mails, or project communications stored in the project folder.
4) **Any changes in accreditation status (if applicable) must be reported to the client either through a telephone call, e-mail communication, or clear reference to the accreditation status in the data report.**

Prior to initiation of work, any differences between the request for services, tender, or contract must be resolved before work commences. The final scope of work, whether a formal contract or informal agreement must be acceptable to both Aquatec and the client. Continuing agreements may not require specific updates in project documents from year to year, however if permit requirements, pricing, or project objectives change, the modifications should be updated and maintained in project files. Relevant communications for on-going projects should be stored in the project folder, electronic files, or phone logs.

4.0 EQUIPMENT AND SUPPLIES

4.1 Equipment Inventory

Aquatec's Master List of major laboratory and field equipment is located in Appendix F. Disposable supplies used for routine analyses are purchased from a reliable supplier such as Fisher Scientific or other suppliers. When materials are received, the shipping package and contents should be examined to ensure that the product has not been damaged.

Major equipment and reference standards (certified thermometers and reference weights) must be properly maintained, inspected, and cleaned at appropriate intervals as specified in SOPs. Maintenance and calibration procedures must be documented in log books or project files. The Master List of field and laboratory equipment will be updated during the annual Quality Systems review. The Master List should include the following information, as a minimum, for each major item of equipment:

Description of equipment, Model Number, serial number (if provided)
Manufacturer
Number of units available

Additional information stored for equipment may include:

Date received
Date placed in service
Current storage or use location
Condition (e.g., new, used, reconditioned)
Date and description of maintenance procedures
History of damage, malfunction, modification, or repair

Records are maintained in the Master Equipment Data Base on the server. This record should be updated as new equipment is acquired or existing equipment is maintained, repaired or taken out of service or on an annual basis. Records for instruments used and calibrated frequently or daily are maintained in the Calibration Logbooks.

4.2 Refrigeration Systems

The following is a list of refrigerators available for cold storage of samples or other materials requiring cold storage:

Make and Model	Description	Quantity
Hobart	Three door refrigerator units	2
Magic Chef	Small refrigerator unit (1 in Sample Mgt., 1 in Tox Lab)	2
Coldspot	Small refrigerator unit (Tox Lab)	1
White Westinghouse	One door freezer unit	1
GE and Maytag	Chest freezer	6

4.3 Information Systems

Aquatec's computer system is a server-based peer-to-peer network with a Dell PowerEdge 2500 SC server as the storage backbone. This server has three partitioned hard drives with a RAID 5 operating system for backup redundancy to minimize the risk of data loss. Each office and Sample Management has at least one Dell Dimension PC or equivalent. Access to the LMS system and project files is controlled through individual user accounts and passwords. Other hardware includes integrated high-speed printers, scanner, and fax machine for preparing hard copies of data and reports.

Most of the data handling, analysis, and reporting are completed using commercially available software. Our proprietary LMS system uses a Microsoft ACCESS platform while other specialized needs are accomplished using Microsoft Word and/or Microsoft Excel. Statistical analysis of environmental toxicology data is generally performed using Comprehensive Environmental Toxicity Information System (CETIS) statistical packages.

4.4 Laboratory Equipment

The quality of laboratory equipment used for analyses can affect the outcome of analyses. Aquatec uses vendors that have proven to be reliable in providing equipment in a timely manner and providing equipment that supports the quality standards required by the methods.

4.4.1 Routine Maintenance of Laboratory Equipment

Aquatec employs preventive maintenance to ensure that instrumentation is operating within normal ranges and also to prevent equipment down time and to help ensure data validity to the best of our ability. General preventive maintenance procedures, many of which are unique to particular instruments, are outlined in each instrument's operation manual and/or laboratory standard operating procedures (SOPs). These documents also assist in the identification of commonly needed replacement parts, so that a supply of these parts can be maintained at the laboratory. It is the Department Manager's responsibility to make sure that the most current operation manuals and SOPs are available to analysts in the laboratory. Analysts perform documented routine maintenance of instrumentation while external technicians may be called in for major repairs or maintenance (e.g. balance calibration).

A calibration and maintenance log notebook is maintained to record routine and non-routine maintenance performed on instruments.

4.4.2 Reference Standards and Calibration Schedules

Reference standards include Class S weights for calibration checks of balances and NIST-traceable reference thermometers for calibration checks of electronic or mercury thermometers. The following instrument calibrations are performed and documented:

a) Analytical balances (daily and annually): checked daily or at the time of use with class S weights that bracket the expected weighing range, documented in the balance logbooks. Balances are serviced by an external certified service technician annually (SOP TOX1-024).

b) Reference Weights (every 5 years): Reference weights used for balance calibration checks are calibrated to NIST traceable standards by an accredited external source such as:

Mettler Toledo, Inc. 1900 Polaris Parkway Columbus, OH 43240 (800) 786-0034 ext. 712	or:	QC Services 8 Smith Street Harrison, ME 04040 (207) 583-2980

The report of certification must be filed in Aquatec's Balance and Temperature Monitoring Logbook. At each certification event, a label will be applied to the

reference weight storage box indicating the date of certification and the date of expiration.

c) Laboratory thermometers and temperature probes (annually): Thermometers and probes are compared to a NIST-traceable reference thermometer, typically in the range-of-use. Records of calibrations and resulting correction factors are maintained in a binder stored in the Project File room (SOP TOX1-025). To meet NELAC standards, each thermometer is required to have an identifying tag and date of calibration check.

d) NIST Traceable Thermometers (every 5 years): Aquatec's ERTCO mercury thermometers, that are used as a reference standard for calibration of laboratory thermometers or probes, are calibrated to a NIST Traceable standard by an accredited external source such as:

Innocal 625 East Bunker Court Vernon Hill, IL Phone: (800) 323-4340	or	QC Services 8 Smith Street Harrison, ME 04040 Phone: (207) 583-2980

The report of certification must be filed in Aquatec's Balance and Temperature Monitoring Logbook. At each certification event, a label will be applied to the certified thermometer indicating the date of certification and the date of expiration. Reference thermometers not re-certified should be labeled as "expired" or "out of service".

e) Refrigerator and incubator temperatures (hourly): These and other temperature-controlled equipment are automatically logged (Scanlink System) and/or manually checked and recorded.

f) Instrument calibrations (daily): Calibration and routine maintenance of supporting laboratory equipment (e.g. meters and probes used for water quality measurements) are documented. For instruments that are typically used on a daily basis (e.g. water quality instrumentation), each calibration event must be documented in the instrument logbook. If an instrument is not used on a particular day, and entry of "not used" should be entered for that day. Some instruments (e.g. balances, drying oven) are used periodically, but not daily. For those instruments documentation of calibration should occur at the time of use.

g) Calibration checks of new volumetric vessels, adjustable pipettes (Quarterly): Records of quarterly calibrations of pipettes and burettes are maintained in the Calibration Log.

h) Verification of light-cycle function (Quarterly): Records of light Cycle verifications and light intensity (foot-candles measured in representative areas) are maintained in the Calibration Log.

Any discrepancies are brought to the immediate attention of the Laboratory Manager and/or Director.

4.4.3 Instrument Calibration

A variety of instruments and wet chemical techniques are available to support microbiological, toxicological, and ecological tests or investigations. Calibration and standardization procedures vary depending on the instrumentation and analytical methodology required for a specific analysis. Calibration is checked on an ongoing basis (e.g. daily or when used) to ensure that the system remains within specifications. If the ongoing calibration check does not meet established criteria, analysis is halted and corrective action is taken. The procedures include assessment of instrument performance, recalibration, reanalysis of check standards, and possibly reanalysis of samples.

Calibration of instrumentation is required to ensure that the analytical system is operating correctly and functioning at the proper sensitivity to meet established reporting requirements. Each instrument is calibrated with standard or prepared solutions appropriate to the type of instrument and the linear range established for the analytical method.

Instrument-specific SOP's discuss in detail how each instrument is calibrated, including frequency for calibration and re-calibration, and the source of the calibration materials. Water quality instrumentation (e.g., pH meters, dissolved oxygen meter, conductivity meter, and salinity refractometer) are calibrated each day that instruments are used and calibration data are recorded in the Calibration Log. Acceptance limits are available for specific instruments within the daily Calibration Log. If instrument calibration values fall outside the specified range, recalibrate or notify the Laboratory Manager or Deputy QAO. The instrument should not be used for data generation until it can be calibrated to expected ranges. If an instrument is taken out of service due to performance failure, this must be documented in the Equipment Maintenance section of the Calibration Log

in the Toxicology Laboratory. Any backup instrumentation brought into service must meet the calibration standards set forth in the instrument SOP or Calibration Log.

5.0 REAGENTS, REAGENT PREPARATION, AND LAB WATERS

Note: Containers holding reagents or materials must be labeled with the identity of the substance. Leaving an unattended, unlabeled material unattended is considered to be an infraction of good laboratory practices. Water is considered to be a reagent (e.g. label as "DI" for deionized water). Empty containers do not need to be labeled.

MSDS sheets for commercial reagents should be stored in the MSDS log available to any analyst using the material.

5.1 Reagent Receipt and Storage

Reagents are chemical and or biological products used to prepare lab waters, calibration standards, reference toxicants, media, or other solutions or laboratory stock solutions. Materials used for preparation of laboratory standard solutions are purchased from suppliers capable of providing certificates of analysis or purity data. In conformance with NELAC requirements, Aquatec will maintain a record of purchased reagents that will be documented as to source, receipt date, lot number, expiration date (where applicable), and disposal date. This information is to be recorded in the Reagent Log that is located at the reception area. Any certificates of analysis must be stored in the file labeled *Reagents: Certificates of Analysis* located in project files for the current year. Any Material Safety Data Sheets that arrive with a new reagent should be read, and should be filed in the MSDS book.

The package is opened and the contents examined. If any products are received in a damaged condition, this is noted in the Reagent Log and the vendor may be requested to provide a replacement. Reference materials, standards, and commercial reagents are also marked with the date of first opening ("opened" followed by initials and date of analyst opening the product). Reagent quality (e.g., as used to prepare reconstituted waters or standards) is assessed through measurements of acceptability (water quality measurements, control performance, standards within expected boundaries).

Each commercial reagent is required to carry an Aquatec label with the following information completed:

Commercial Reagent Label

Date Received:	Source:
Lot #:	Date Opened:
Storage:	Expiration date:

When the product is depleted (empty bottle) or expired, the discard date / initials should be entered in the final column of the Reagent Log. Containers with expired reagents should be labeled as "expired" and removed from the supply of useable reagents.

5.2 Reagent Preparation

The Laboratory Reagent Preparation Log is used to document use of reagents in preparing working solutions in the laboratory. Stock solutions are prepared by dissolving known amounts of reference materials, standards, or commercial reagents in a suitable solvent (usually de-ionized water). Each laboratory-prepared standard or solution (prepared reagent) in the laboratory must be linked to the original reagent through the lot number of the original reagent(s). The lot numbers of the original purchased reagents must be recorded in the Reagent Preparation Log at the time of preparation, as well as dates of expiration (if applicable).

Each prepared reagent is required to carry an Aquatec label with the following information completed:

Prepared Reagent Label

Description / Use:	
Date prepared:	Lot #:
Storage:	Expiration date:

Reference toxicant solutions must also carry linkage to the lot numbers of stock solutions (recorded on SRT sheets). The lot number of reference toxicant solutions may be the sample number, assigned during login or a date of preparation followed by the toxicant identification.

Prepared reagents must meet the quality standards of the applicable test method. For example, when reagents are used to prepare reference toxicant solutions, the response pattern should fall within the control chart limits at least 95% of the time and water quality measures must be within acceptable ranges.

5.3 Reagent Grade Water and Laboratory Waters

Several types of water are used at Aquatec for the many types of toxicity tests that are performed in our laboratories. SOP TOX1-018 defines the quality control checks for these waters. Preparation of laboratory reconstituted waters must be documented in the Water Preparation Log and the required water quality parameters must be measured and documented before first use.

The following is a description of our various types of water:

NanoPure water: our highest level of water purity with a resistance of at least 18megOhm/cm after passing through the NanoPure filter apparatus. This is used as the base water for reconstituted waters, reagents, and media.

De-ionized water: water that has passed through our pre-filtration, organic carbon, de-ionizing resins, and UV treatment. This is used to prepare some reconstituted waters and for final rinsing in glassware and plastic-ware cleaning.

Tap water: untreated water, directly from the city-serviced tap. This is used for detergent washing glassware and plastic ware and as a warming medium when warming toxicity test samples to temperature.

Soft water: reconstituted water used as laboratory water controls and dilution water for some toxicity tests.

Moderately hard water: reconstituted water used as laboratory water controls and dilution water for some toxicity tests.

Hard water: reconstituted water used as laboratory water controls and dilution water for some toxicity tests.

Reconstituted water for freshwater sediment tests ("Sed Recon Water"): reconstituted water used as overlying water for freshwater sediment toxicity tests.

Forty Fathoms: artificial seawater prepared by adding Crystal Marine Mix salts to de-ionized or NanoPure water to the specified salinity.

Sea water: natural seawater collected from a shore zone. Used as dilution water for some toxicity tests (e.g., *Arbacia punctulata* fertilization).

Lake water: Lake Champlain water used in proportion with moderately hard water for *Ceriodaphnia dubia* cultures and Sediment Recon Water for *Hyalella azteca* chronic sediment toxicity tests. Filtered (e.g., 60 micron or less) before use.

The quality of standard dilution water is assessed through routine chemical measurements (pH, dissolved oxygen, conductivity, salinity, alkalinity, hardness) and also through support of acceptable survival, growth, or reproduction in control treatments associated with toxicity tests. Also, analysis of laboratory water (e.g., soft water, moderately hard water, or Forty Fathoms artificial seawater) is performed quarterly if it is used as dilution water related to permits requiring chemical characterization of dilution water. Parameters typically monitored on a quarterly basis for lab waters used as dilution water include: TS, TDS, TOC, TRC, total ammonia, and metals.

5.4 Laboratory Water Preparation

Preparation of laboratory waters must be documented in the Water Preparation Log using the ingredients and instructions specified in the Water Preparation Log for each specific type of water. Each batch of laboratory water should have reference to reagent lot numbers and an assigned lot number for that batch of laboratory water (consisting of date prepared followed by type of water, e.g., 040808FF).

The prepared water must be labeled with the date and initials of the analyst preparing the water. The prepared water should be aerated at least overnight and then characterized (water quality parameters) before first use. Typically laboratory waters expire within two weeks of preparation.

6.0 PROCEDURES FOR HANDLING SAMPLES

Sample integrity is the foundation upon which meaningful biological results rely. Occasionally clients request that Aquatec personnel conduct on-site sampling. For these projects, an approved Project-specific Sampling Plan or Sampling and Analysis Plan (SAP) should be developed. In lieu of a formal SAP, a project-specific standard

operating procedure (SOP) may fulfill requirements for a sampling plan. The SAP or SOP should define the data quality objectives relevant to the assessment goals specific to the sampling site.

For most samples received at Aquatec, it is the responsibility of the client to deliver samples within specified holding times and under appropriate storage and shipping conditions. The integrity of samples must be maintained through the use of appropriate sample preservation techniques as specified in project-specific protocols or work plans and Aquatec's Sample Acceptance Policy.

Samples delivered to the laboratory are logged in as soon as practical, typically upon delivery. Any sample that is suspected of being contaminated, improperly stored or preserved, or improperly prepared, is documented and included as a "qualifier" in the report. Instances where sample condition varies widely from normal are reported to the Department Manager and/or client. Guidance for storage and final disposal of samples is located in Appendix B of this QAPP. An example of a project-specific sampling plan is outlined in SOP TOX1-026.

6.1 Chain-of-Custody

The critical nature of Chain-of-Custody (CoC) procedures cannot be overemphasized. These procedures generate a historical record of sample custody from the time of acquisition to time of completion of the analysis. In most cases, samples are stored beyond the analysis time and then discarded following project-specific guidelines or in-house protocols for sample disposal (Section 10 and Appendix B to this QAPP).

The Chain-of-Custody procedures employed at Aquatec are implemented through our Laboratory Management System (LMS) or with client-generated Chain of Custodies. An example Chain-of-Custody form is presented in Figure 4 (Appendix E). The following procedures have been established to ensure samples are secure and properly stored.

- Clients sending samples should be instructed as to the necessity of completing the Chain-of-Custody form and sample labels.
- Visitors or delivery personnel are greeted at the reception area and laboratory personnel sign to acknowledge receipt (commercial carrier). Commercial carrier personnel (e.g. Fed Ex, UPS, and Priority Express) are not required to sign the Chain-of-Custody documentation since the coolers are typically sealed when they arrive. Delivery personnel are restricted from laboratory areas unless escorted by laboratory personnel.

- Samples remain in sample storage until removal for sample preparation or analysis.
- While not commonly requested, transfers of samples into and out of the storage area(s) can be documented on an Internal CoC record. The assigned sample custodian, or his designate, will control the internal custody of samples.
- After a sample has been removed from storage for analysis, the analyst is responsible for returning the sample to the storage area before the end of their working day.

6.2 Sample Receipt and Sample Acceptance Policy

SOP TOX1-017 is a companion document containing additional information regarding sample acceptance. Only authorized Aquatec personnel receive samples at the laboratory. Samples typically arrive in coolers with thermal preservation (iced). The cooler is opened and a thermometer is placed inside the cooler (preferably into a Temperature Blank that was placed inside the cooler with the samples) to measure the ambient temperature. The ambient temperature is recorded on the CoC form. Some samples do not require thermal preservation (e.g., macroinvertebrate samples are typically preserved with formalin or ethyl alcohol, samples for metals analysis (subcontracted) or total ammonia are preserved with acid). The CoC is signed including date and time of receipt by the personnel who receives and handles the cooler and samples. Samples are removed from the cooler and sample labels are compared with the sample descriptions on the CoC form. Each sample is assigned a unique laboratory identification number (sequential six-digit number) using Aquatec's LMS and logged in for the requested parameter(s). The sample number is maintained electronically (Access data base) and on paper in the form of work sheets which specify the requested analyses. The analyst must provide initials on the login form. A sample number label is also physically affixed to the sample container(s). In many cases, several containers of the same sample are received (e.g. several 1-gallon containers and chemistry bottles for an effluent sample). In these cases, the sample in the multiple containers is logged in as a single sample and each container of that sample would receive the same sample number. The sample number is the key mechanism for tracking any sample throughout the laboratory. Any deviations from normal conditions (broken or uncapped containers, temperature out of range, samples beyond holding time) should be documented on the Chain-of-Custody form or on log-in sheets that accompany the samples for analysis.

We provide information to our clients regarding the proper sample shipping conditions (e.g., footnotes on Chain-of-Custody forms provided to clients). While most samples arrive within the specified conditions, occasionally samples arrive outside the target boundaries (e.g., samples that are recently collected may not have cooled to 6°C when they are delivered to us.). Discrepancies in sample condition must be documented and the Laboratory Manager should be notified so that possible corrective action can be initiated. If corrective action is activated, a description of the corrective action should be provided on a "Corrective Action Report" (Appendix E). The resolution of discrepancies will be noted either on the CoC record, the log-in sheet, or the Corrective Action Report stored in the project file. For example, if the cooler temperature exceeds 6°C, the Laboratory Manager or the Director will make a decision as to whether the client should be notified to warn of the discrepancy. Communication with clients regarding potential breach in sample integrity (e.g. too warm, broken sample containers), can take the form of documented phone calls, Fax, or e-mail, and client's authorization to proceed with the analysis. Many biological analyses are time-sensitive (i.e. tests must be started within holding times) and in some cases the client contact is not available. Therefore, it may be more important to proceed with the analysis and report the result as "qualified" rather than stall the analysis for unresolved sample condition issues.

If the sample does not meet sample receipt acceptance criteria, correspondence or records of final disposition of rejected samples should be documented and stored in the project file. Reports for analyses that proceed with samples not meeting acceptance criteria must be qualified by recording the condition of the samples on the chain-of-custody form or associated paperwork. This documentation should be included in the report package, with appropriate qualifiers included in the report.

Aquatec utilizes the LMS to track samples and analytical data. These data include:

- Sample number (unique to each sample)
- Date received
- Initials of analyst logging in the samples
- Sample descriptions or IDs
- Date and time of collection
- Additional comments (e.g. non-compliant conditions)
- Client name
- Client address
- Client project number
- Notation of any special handling instructions or priority assignments

- Analyses requested

6.3 Biological Test Request

The information listed above becomes embedded in the Biological Test Request (BTR). The BTR and the unique sample number are generated in the Laboratory Management System for each sample or series of samples. The BTR contains all of the specific information relative to a particular sample or group of samples. BTR worksheets are printed from LMS and are also stored electronically. The BTR worksheets accompany the sample during the analysis and are maintained in the project file along with the data generated during the analysis and the final report. The LMS allows us to track samples from log-in through analysis, reporting, and final invoicing. The method-specific analytical worksheets for a BTR provide analyst(s) with a means to generate and print project-specific bench-sheets through the LMS system.

6.4 Test Identification

During the login procedure, along with unique sample numbers and BTR number which are generated automatically, a unique test identification number for each test method is also generated. This number is associated with test-specific bench sheets and statistical analysis linkage codes.

6.5 Sample Delivery Group

Sample Delivery Groups (SDGs) link a series of BTRs together for reporting purposes. Often a series of samples, delivered on different days, are required to complete an analysis (e.g., for chronic whole effluent toxicity tests where renewal samples are required) or to meet the experimental design requirements (e.g., for sediment toxicity tests where a series of samples are tested or reported concurrently). The BTRs are assigned to an SDG, defined as the first BTR number assigned for any group of samples being linked in this way. Typically the first BTR number for a series of related samples is also assigned as the SDG number in the LMS system.

6.6 Sample Matrix Changes

Some laboratory procedures result in the generation of new samples from treatments or manipulations of the original samples. While the original samples retain their assigned sample numbers, new samples or matrices should be logged in and assigned sample numbers. Examples of this situation include extraction of pore water from sediments,

preparation of elutriates from mixtures of sediment and water, generation of tissues from bioaccumulation tests. In most cases it will be important to retain client sample identifications along with a suffix (e.g. -PW for pore water, -Mn for *Macoma nasuta* tissue) added as a tag to the original identification. Clear linkages to the original samples must be provided.

6.7 Subcontracted Analyses

Some samples require analyses that are not routinely performed at Aquatec (e.g. chemical analyses). These analyses may be subcontracted to qualified laboratories for the specific analysis. When appropriate, the sub-contract laboratory may be assessed by the laboratory either through an on-site visit or by the submission of sufficient documentation to determine the sub-contractor's capabilities and qualifications. As a minimum, certification is required from NELAC and/or the State where the samples originated. Other required documentation may include laboratory QAPP, SOP's, recent PT sample results, or other relevant certifications. Samples being analyzed for NELAC-accredited parameters should be sent to a laboratory that is NELAC-accredited for the required parameters.

Samples for subcontracted analyses are shipped to laboratories under CoC protocols. Reports received from subcontracted laboratories are reviewed at Aquatec and incorporated into the final report sent to the client. The client is advised of the subcontracted laboratory and their accreditation status by inclusion of the analytical report within the body the report generated at Aquatec.

7.0 SAMPLE ANALYSES, REVIEW AND REPORTING

Detailed descriptions of procedures for the analyses of samples are maintained in laboratory method-specific SOP's. Appendix C of this QAPP presents a summary of the methods employed by Aquatec. Documentation of observations, environmental conditions, and data associated with analytical methods and procedures must be recorded on bench sheets or project notebooks at the time the observation is made, along with the initials and date (and possibly time). Reviewers and clients should be able to assess whether the data supporting the analysis are clear and complete.

Preliminary measures may be required to assess the suitability of samples for the requested analysis. For example, before initiating toxicity tests, water quality parameters (e.g. temperature, pH, conductivity, dissolved oxygen, salinity, and total residual chlorine) should be measured and documented.

In general, departures from SOP-driven procedures or from standard or published methods are not permitted. However, project-specific objectives may require method development or specialized sample manipulations. In cases where a procedure is modified from a standard protocol, the modifications will be documented in a project-specific SOP or described in a narrative or qualifier which accompanies the analytical report.

7.1 Guidance Documents for Analytical Methods

The following documents describe analytical methods performed at Aquatec and they are the basis for our method-specific SOPs:

EPA-821-R-02-012, *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms*, 5th Edition October 2002.

EPA-821-R-02-013, *Short-Term Methods For Estimating The Chronic Toxicity Of Effluents And Receiving Water To Freshwater Organisms*, 4th Edition, October 2002.

EPA-821-R-02-014, *Short-Term Methods For Estimating The Chronic Toxicity Of Effluents And Receiving Water To Marine And Estuarine Organisms*, 3rd Edition, October, 2002.

EPA 821-B-00-004, *Method Guidance and Recommendations for Whole Effluent Toxicity Testing (WET) Testing*, July 2000.

EPA 833-R-00-003, *Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications Under the National Pollutant Discharge Elimination System.* June 2000.

EPA 600/R-99/064, *Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates*, Second Edition, 2000.

EPA 600/R-94/025, *Methods for Assessing the Toxicity of Sediment-associated Contaminants with Estuarine and Marine Amphipods*, 1994.

EPA 600/4-90/030, *Macroinvertebrate Field and Laboratory Methods for Evaluating the Biological Integrity of Surface Waters*, 1990.

EPA 841/B-99/002, *Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish*, Second Edition, 1999.

EPA 600/4-79/020, *Methods for Chemical Analysis of Water and Wastes*, 1983.

EPA 600/R-93/100, *Methods for the Determination of Inorganic Substances in Environmental Samples*, August 1993.

EPA SW-846, *Test Methods for Evaluating Solid Waste, Physical/Chemical Methods*, 3rd Edition, with Update I, Update II, and Update IIA, September 1994.

EPA / USA CoE NED, *Regional Implementation Manual for the Evaluation of Dredged Material Proposed for Disposal in New England Waters*, April 2004

EPA 823-B-98-004 *Evaluation of Dredged Material Proposed for Discharge in Waters of the U.S. – Testing manual, Inland Testing Manual*, 1998

EPA / A CoE, *Evaluation of Dredged Material Proposed for Ocean Disposal (Testing Manual)*, 1991

EPA/600/6-91/003; *Methods for Aquatic Toxicity Identification Evaluations, Phase 1 Toxicity Characterization Procedures*, 1991

EPA/600/6-91/005F; *Toxicity Identification Evaluation, Characterization of Chronically Toxic Effluents, Phase 1*, 1992

EPA/600/R-92/080; *Methods for Aquatic Toxicity Identification Evaluations, Phase 2 Toxicity Identification Procedures for Samples Exhibiting Acute and Chronic Toxicity*, 1993

EPA/600/R-92/081; *Methods for Aquatic Toxicity Identification Evaluations, Phase 3 Toxicity Confirmation Procedures for Samples Exhibiting Acute and Chronic Toxicity*, 1993

EPA/600/R-96/054; *Marine Toxicity Identification Evaluation (TIE), Phase 1 Guidance Document*, 1996

APHA/AWWA/WPCF, *Standard Methods for the Examination of Water and Wastewater*, 19th Edition.

ASTM, *American Society for Testing & Materials; Annual Book of Standards*, 2004. Volume 11.05 Biological Effects and Environmental Fate; Biotechnology; Pesticides.

ASTM, *Standards on Materials and Environmental Microbiology*, 2nd edition. 1993.

NOAA (National Oceanic and Atmospheric Administration), *National Status and Trends Program*, Volume I-IV, 1984-1994.

Plumb, Russell, USEPA Corps of Engineers, *Procedures for Handling and Chemical Analysis of Sediment and Water Samples*, May 1991.

FDA, *Bacteriological Analytical Manual* (BAM), 8th edition. 1995.

AOAC, *Official Methods of Analysis*, 14th edition. 1984.

7.2 Data Reduction

Each Department provides data review prior to reporting results to the client. In general, an analyst will process data in one of the following ways:

- Manual computation of results with manual reporting
- Computer computation of results with manual reporting
- Computer computation and reporting of results

If the analyst manually processes the data, steps in the computation are provided for review including the source of the input parameters such as response factors, dilution factors, and calibration constants. Calculations of manually processed data are checked during review.

For data that is processed using a computer and then entered into the LMS or statistical programs, a hard copy of the computer-generated results is provided with any other preparation or dilution information as may be needed.

If computer-processed data are directly acquired from instrumentation, hard copies of the actual data are printed and the analyst verifies that the following are correct before releasing instrumental data to the reporting system:

- sample numbers
- calibration constants/ response factors

- output parameters such as units and identifiers
- dilution and preparation factors

The hard copy of the results is used for data validation and review. After initial demonstration of proficiency of computerized programs, computer calculations are periodically spot checked for consistency and accuracy. Commercially available statistical programs utilized in data analysis are checked annually using a known data set.

7.3 Data Review

A documented review of data and analyses supporting a client report is performed before sending the report to the client. This review incorporates an evaluation of sample receipt, sample preparation, test conditions, supporting analyses, data anomalies, and QC standards associated with the results of a particular method.

The analyst is responsible for preliminary review of data generated in the laboratory. If the instrument calibration and/or quality control samples (e.g. toxicity test controls) are within specified tolerances, then the data are presented to data reviewers for secondary review. If instrument calibration or the quality control samples (e.g. toxicity test controls) exceed specified tolerances or acceptance limits, then affected sample results are evaluated and the samples may be submitted for re-analysis, a re-sampling may be requested of the client, or the results may be submitted as "qualified". If discrepancies or deficiencies exist in the analytical results, then an appropriate corrective action may be required.

A secondary review is conducted by Laboratory Managers or designated analysts who review the bench-sheet data to determine whether analytical results are acceptable. Also, calibrations, manual calculations, and transcriptions are checked for accuracy and completion of initials, dates, and times. Quality control sample results are evaluated against the specified acceptance criteria. If instrument calibration and quality control samples are acceptable, then the data may be prepared for reporting.

After analytical data have been reviewed, the final report can be assembled for submission to the client. The completed report and supporting data package are reviewed to verify that the results are presented in a clear, correct, and concise manner.

7.4 Data Reporting

The Director/QAO and Laboratory Managers have authority to approve and release data and reports to clients once it is certain that the reported data and supporting documentation accurately reflect the completed work. To the extent possible, results are reported only if QC measures are acceptable or when appropriate qualifiers have been incorporated into the report.

Aquatec reports data associated with biological analyses. Typically reports do not fit within the strict definitions of Levels 1-5 common to analytical chemistry laboratories. Our data are reported within the general provisions of Level 2 (e.g. microbiology and ecology reports) and Level 4 (e.g. microbiology, ecology, and toxicity reports). Field measurements generally meet Level I data requirements.

The various data/reporting categories (US EPA) are as follows:

Levels 1 and 2 data consists of measurements with the report consisting of results only. Field or microbiology data may be reported as Level 1 data without supporting documentation. Any supporting documentation or related quality-control data must be archived in project files along with the report.

Level 3 reporting consists of an analytical report with internal quality control results reported; these include laboratory control standards, surrogate spike recoveries, and method blank results. Reports incorporating supporting analytical measures (such as total ammonia) would include the QA/QC data.

Level 4 data refers to data submitted in CLP-like format. Level 4 is defined by the submission of QA/QC supporting data and raw laboratory data (recorded on bench sheets). Submission of data in this format results in a package that can be independently validated. Level 4 reporting includes a QA narrative, analytical results, qualifiers, supportive documentation including raw data and preparation sheets, and documentation related to Chain-of-Custody. Once the document is assembled, the report sections are distinguished with title pages. Copy(s) of the documentation are sent to the client either as an electronic file (PDF) or as a paper copy. The original document is retained at Aquatec project files for a minimum of five (5) years.

Level 5 data has unique requirements in compound identification, quantification, detection limits, clean-up or QA/QC requirements. This level of reporting is not typical of the biological analyses performed at Aquatec.

Analytical results that are transmitted by phone, facsimile, or electronically (e-mail) should be done only at the request of a known and appropriate representative of the client. For microbiology results, a pre-arranged and paid agreement is requested of the client and this is documented on the microbiology analysis Chain-of-Custody form. For other analytical results submitted by facsimile or e-mail, the facsimile form should contain a confidentiality statement. Occasionally, known clients wish to be apprised regarding the progress or outcome of analyses. In these cases, the analyst or project manager should be reasonably sure that the correct client contact is being informed and unless the data or the analyses have gone through final review, the information should be presented as "preliminary".

Reports are stored both as paper originals (in project folders) and are duplicated electronically as secure (un-editable) PDF files within the computer-based project folder.

7.4.1 Standard Report for Toxicology Studies

The standard report typically contains the following elements:

- Title;
- Aquatec Biological Sciences, Inc. (for analyses conducted here);
- Identification of laboratories used for any subcontracted analyses;
- Unique identification of the test report (SDG number, sample numbers);
- Project name and name and address of the client;
- Test method identifications and descriptions of test conditions (method summary);
- Pagination for multiple page reports;
- Cover letter

Any opinions or interpretations of test data are presented as such, with documentation provided to support such opinions or interpretations.

Subcontracted services or reports for client services are identified by inclusion of the entire report received from the subcontracted laboratory.

The body of toxicity reports contain tabulated data in the form of a Summary Report; Detail Report (e.g. replicate data), and a Quality Control section. The Quality Control section incorporates calculated values of data quality indicators (i.e. control treatment performance specific to the test method) and a discussion of any qualifiers for the reported results, including any events that may cast doubt on the validity or usability of

the data. Reports for sediment evaluations include a Quality Control Summary Table with data quality indicators and test acceptance criteria clearly identified.

To the extent possible, results are reported when the QA/QC measures fall within applicable ranges of acceptability. In some cases, however, results may be reported with appropriate qualifiers when acceptance criteria are not met. If any aspect of the work does not conform to method protocols or QA/QC standards, the non-conforming data must be presented within the body of the report. Also, the QA/QC narrative should describe the non-conforming event(s) and suggest appropriate corrective action, if needed, to meet regulatory requirements.

In some cases, state, local regulators, or clients may require or request a reporting form that is specific to a particular region. In these cases, while the format of the report may vary, all of the elements listed above are included within the body of the report and copies of supporting data (bench sheets) are included.

Reports and associated data or amendments to reports are stored both as paper originals and as secure electronic files (PDF).

7.5 Report Revisions

Amendments or corrections to reports are identified by reference to the original analysis (e.g. sample number and SDG) along with justification or explanation of the correction. If it becomes necessary to issue a revised or complete new report, the revised or new report should be uniquely identified and contain reference to the original report.

8.0 QUALITY CONTROL ELEMENTS

8.1 Laboratory Water

The quality of our base water and reconstituted waters is of primary importance to our functioning as an aquatic laboratory. Aquatec has a water-quality monitoring program in place to insure that high quality standards are met. Details of the monitoring program are outlined in SOP TOX1-018. As a minimum, our base water (de-ionized water used for final rinses of cleaned glassware and for preparation of reconstituted waters, and NanoPure water used for making reagents and media) will be monitored annually for organics (pesticides and PCBs) and metals as specified in EPA-821-R-02-012/013/014 series documents. Total Kjeldahl Nitrogen (TKN) and total organic carbon (TOC) are also included in the annual analysis of NanoPure water. Our NanoPure is also

monitored monthly for total residual chlorine (TRC), pH, and total bacteria. Reconstituted water (e.g., soft water), when used for dilution water in toxicity tests are monitored for TRC, total ammonia, total and dissolved solids, total organic carbon, and metals quarterly. Samples associated with this monitoring program are submitted to a laboratory certified for the requested analysis. Results of the analyses (analytical reports) are reviewed and archived for a minimum of five years.

The water purification system consists of particle removal pre-treatment (2), carbon bed (1), reverse osmosis (R/O) membrane (2), de-ionizing resin beds (2), 300 gallon storage tank with a continuous circulation delivery loop powered by a Dayton 6K578C pump. Higher levels of water purification are attained by passing R/O de-ionized water through the NanoPure system, resulting in 18.3 megohm-cm quality water.

Maintenance schedule for water purification system:

- Daily: NanoPure quality check: allow to run ~1 min. and record meter reading in the Toxicology Laboratory Daily Calibration Log;
- Daily: check the warning light on the de-ionizing resin beds. If the light goes to red, notify the Laboratory Manager or the Director. Replacement of one or both resin beds may be required.
- Weekly: record pressure and performance parameters on the weekly check list. If anything appears to be abnormal, notify the Laboratory Manager or the Director.
- Every three months or as needed based on monthly heterotrophic bacteria counts (<1000 CFUs/mL): replace pretreatment Filter 1 (DGD-2501-20 25 micron), pretreatment Filter 2 (EP-20BB carbon block) and Filter 3 (GE Merlin 10 micron R/O sediment pre-filter).
- Every six months, or as needed: replace NanoPure D0749 (Fisher 0903449) 0.2 micron filter
- Every 18 months or as needed based on purity level of greater than 18 megohm-cm: replace NanoPure D0835 (1 pre-treatment); D0809 UltraPure (2)
- Every 6 months or as needed (based on warning light): de-ionizing resin beds
- Annually: replace UV system lamp

Records of the DI System maintenance are kept in the DI system/Nanopure Record Book.

The quality of reagent or laboratory water is also assessed through satisfactory survival, growth, and reproduction of test species as demonstrated by routine reference toxicant tests and control responses.

8.2 Organism Receipt

Specific instructions for receiving, acclimation, and holding of organisms for toxicity testing are included in method-specific SOPs. The apparent condition (e.g. "normal") of organisms along with measured environmental parameters must be documented on the information sheet received with the organisms. Batches of organisms must be identified as to species code, receipt data, and project assignment on the holding container. Any water changes or feeding activities should be documented either on the organism information sheet or a species-specific "holding and acclimation" data form. Guidance for acceptance or rejection of organism batches are included in method/species specific SOPs. Organisms of a particular batch must be stored in uniquely separated containers so as to avoid any chance of cross-mixing with other batches of the same or different species. Organisms for one test must be from the same batch.

8.3 Food Stocks

Organisms used in toxicity tests are expected to be healthy and nutritionally satisfied before and/or during testing. Food stocks used for organisms in toxicity tests must be administered at a quantity as specified by the protocol and the quality of the food is assessed through comparisons of laboratory control responses to EPA acceptance criteria.

Artemia (brine shrimp) eggs are purchased from a commercial supplier. Successful hatchability and support of chronic toxicity tests for minnow larvae will be assessed through survival and growth (average dry weight per surviving minnow) in controls based on EPA criteria: Laboratory control must meet or exceed 80 percent survival and 0.25 mg average dry weight per surviving minnow for *Pimephales promelas* and 80 percent survival and 0.5 mg per surviving minnow for *Menidia beryllina*).

Food stocks used for culturing the water flea, *Ceriodaphnia dubia, Daphnia pulex, or Daphnia magna*, include the alga, *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*), and Yeast-Cerophyl-Trout Chow (YCT) obtained from a commercial supplier such as Aquatic Biosystems of Fort Collins Colorado. Chemical analysis profiles of YCT are provided by the supplier.

Pseudokirchneriella subcapita is stored refrigerated with a shelf life limited to two weeks. YCT if stored refrigerated should be used within 10 days. If immediately frozen upon receipt, it may be stored three months, then thawed and used within 10 days. These stocks are used to maintain the brood boards leading up to toxicity test use and also to feed test organisms. The *Ceriodaphnia dubia* brood boards are tracked for survival and reproduction daily during the week preceding the use of their offspring for testing. On-going toxicity test laboratory control performance also provides on-going monitoring of the quality of food stocks. Controls must meet the minimum acceptance criteria of 80% survival and an average of 15 or more neonates per surviving female. It is during this time that the quality of the food stocks is verified. If a depression in survival or reproduction appears to be linked to a particular batch of food, a new batch is acquired as soon as possible, and the old batch is discarded.

In-house cultures of the sea urchin, *Arbacia punctulata*, are fed fresh Romaine lettuce acquired from local grocery stores. This food source has been adequate to support gravid adult sea urchins for years at Aquatec.

8.4 Data Quality Objectives

The data quality objectives discussed below ensure that data will be gathered and presented in accordance with procedures appropriate for their intended use, and that the data will be of known and documented quality able to withstand scientific and legal scrutiny. The quality of the measurement data can be defined in terms of precision, accuracy, representativeness, completeness, comparability, and traceability.

8.4.1 Precision

Precision is the determination of the reproducibility of measurements under a given set of conditions, or a quantitative measure of the variability of a group of measurements compared to their average value. Precision is typically measured by analyzing field duplicates and laboratory duplicates (sample duplicate, matrix spike duplicate, and/or laboratory duplicate). Precision is most frequently expressed as standard deviation, percent relative standard deviation, coefficient of variation, or relative percent difference. Precision goals are specified in most methods. Whole effluent testing protocols typically require within-test replication, resulting in a measure of precision inherent to the test and reflected in the statistical analysis. Precision measures may not be applicable to some biological analyses.

Some regional agencies may require a discussion of percent minimum significant difference (PMSD) in the report. PMSD is a measure of the within-test variability, which affects the sensitivity of the test data. Evaluation of concentration-response relationships may also be required. While guidance for these discussions are provided by US EPA (EPA 821-B-00-004 and EPA 833-R-00-003), there may be a level of best professional judgment applied to these discussions.

8.4.2 Accuracy

Accuracy is the measure of agreement between an analytical result and its "true" or accepted value. Deviations from a standard value may result from a change in the measurement system. Potential sources of deviations include (but are not limited to) the sampling process, sample preservation, sample handling, matrix effects, and sample analysis. Analytical laboratory accuracy is determined by comparing results from the analysis of matrix spikes, surrogates, or check standards to their known values. Accuracy results are generally expressed as percent recovery, acceptance ranges, or accuracy goals may be specified in methods. Accuracy measures may not be applicable to some biological analyses.

For toxicity analyses measures of accuracy, an assessment of our performance with unknown samples is generated through participation in annual US EPA DMR studies. Our performance in these studies is made available to appropriate NELAC personnel for accreditation purposes and to regulatory agencies or clients requesting the results.

8.4.3 Representativeness

Representativeness of a sample is determined by the sampling personnel in collecting a representative sample and the laboratory using a representative portion of the sample for analysis. The laboratory makes every effort to ensure a representative aliquot is removed from the sample container. Homogenization of the sample (e.g. for sediment samples) may be carried out in the laboratory when appropriate.

8.4.4 Completeness

Completeness is defined as the percentage of measurements that are judged to be valid. Factors negatively affecting completeness include the following; sample leakage or breakage (in transit or during handling), violation of specified holding times, loss of sample during laboratory analysis through accident or improper handling, improper documentation such that traceability is compromised, or rejection of sample results due

to failure to conform to QC criteria specifications. A completeness objective of at least 90% of the data is the goal established for most projects. Aquatec strives for a completeness goal of 100%.

8.4.5 Comparability

Comparability of results between current and past sampling events, and between analytical sequences of a method is achieved through Quality Assurance Project Plans (QAPP), controlled SOP's, regulatory protocols, and experienced well-trained analysts.

8.4.6 Traceability

Traceability is the extent to which reported supporting documentation can substantiate analytical results. Traceability documentation exists in two essential forms: those that link the quantification process to authoritative standards, and those that explicitly describe the history of each sample from collection through analysis. Aquatec strives for a traceability goal of 100%.

9.0 QUALITY CONTROL MEASURES

The quality control program implemented at Aquatec includes the analysis of check standards and appropriate control treatments. Analytical sample series include controls specific to the analysis. The controls used in an analysis should be completely representative of the procedure, including sample preparation and sample analysis.

The following describes procedures used to monitor method performance and support data validation. Control treatments analyzed in conjunction with samples are essential to the evaluation of the data quality. The following quality controls may be incorporated into specific analytical procedures, biological tests, or investigations.

9.1 Method Blanks

Method blanks are typically prepared and analyzed with an analytical batch of twenty or fewer samples to identify possible sources of contamination within the analytical process, however, method blanks may or may not be relevant to some analyses conducted at Aquatec. Method blanks, if applicable, are treated as samples (i.e. they must go through each stage of the analytical process), including glassware, reagents, instrumentation, exposure, and any other source of possible contamination that may affect sample results.

For some biological analyses, the method blank may be represented by the negative control (e.g. whole effluent toxicity tests). For other biological analyses such as bioaccumulation tests, a representative sub-sample of the test organisms may be submitted to the analytical laboratory to assess "background conditions" of the organisms. If applicable, the acceptance limits for method blanks are defined in the method SOP's.

9.2 Laboratory Control Samples (negative controls)

Laboratory Control Samples (LCS or "Control") are used to assess the acceptability of toxicity tests and provide information to assess the validity or usability of test data. Control samples typically prepared and analyzed with each batch of samples (samples tested concurrently) or with individual samples. In the Toxicology Laboratory, aqueous and/or solid (sediment or soil) laboratory control samples are analyzed using sample preparation, environmental conditions, and analytical methods representative of the methods employed for the samples received. Control samples are typically incorporated within each test method for a group of samples. The control limits and corrective actions for Controls are defined in the method SOP's and relevant guidance documents. See also Section 9.10 of this document for additional information regarding toxicity test controls.

9.3 Laboratory Control Samples (positive controls)

Positive controls for the Toxicology Laboratory are represented by the Standard Reference Toxicant (SRT) tests that are routinely performed with test organisms. In this quality control program, a known toxicant is prepared, and a reference toxicant test is initiated with a representative sub-sample of the test organisms. A species should respond in a consistent way to a presented toxicant when tested repeatedly over time and under similar conditions (age and environmental). Initial limits are established after a minimum of five data points. Once established, the control limits are updated whenever more data points are generated, and the control chart is maintained based upon the most recent 20 values. These control limits, once calculated, are used to monitor the quality of test organisms and laboratory techniques (See also Section 9.10).

9.4 Replicate Analyses

Replicate analyses (e.g. field or laboratory duplicates) are often received with groups of samples requested for analyses. These client-provided duplicates are typically treated as an independent sample and are included in the concurrent testing group, when

requested by the client. Toxicity testing protocols include laboratory replicates (of each sample or treatment level) inherent to the test method, as outlined in the guidance documents and Aquatec SOPs for the individual tests.

9.5 Calibration Check Standards

In the Toxicity Laboratory, calibration check standards are used daily to calibrate and evaluate the performance of water quality instrumentation.

9.6 Statistical Software Quality Control

Aquatec currently utilizes the Comprehensive Environmental Toxicity Information System (CETIS) statistical programs for evaluation of toxicity data.

As part of our annual Quality System Review, Aquatec will enter a known data set into software programs. For example, a data set from US EPA toxicity testing guidance documents may be used to assess the function of CETIS. This annual assessment of the performance of statistical packages will be documented, reviewed, and filed. If acute or chronic values match published values (within the confidence limits), the statistical package will be assessed as acceptable. Our CETIS statistical package is also tested annually through the US EPA DMR Quality Assurance study because this statistical package is used to generate the values submitted for this study.

In-house statistical or calculation programs developed by Aquatec will also be assessed annually completing an independent computer-based or manual calculation to verify that the program is functioning properly. The results of this assessment will be stored in project files.

9.7 Equipment Quality Control

9.7.1 Volume Calibration Checks

Pipettors are checked quarterly for volume accuracy. Adjustable and non-adjustable volumetric pipettes and burettes are checked for accuracy at least quarterly using volumes normally used. The measured value (based on weight of deionized water) should be within 5% of the expected volume. For volumetric pipettes, tare a small cup or weigh-pan on the balance, pipette the volume of de-ionized water into the cup. The volume should equal the weight, grams = mL ± 5%. If a pipettor does not meet this criterium, it should be re-checked to determine whether an error in weighing had been

made or whether the pipettor is out of the acceptable calibration range. The results are recorded in the Pipette calibration section of the Calibration Log Book. Pipettors that do not meet the 5% criterion are removed from service or sent out to be repaired.

9.7.2 Autoclave Operation

Records of autoclave operations are maintained for each cycle, including: date, contents, maximum temperature reached, pressure, time of sterilization mode, total run time, sterility tape, and analyst's initials.

9.8 Standard Reference Toxicant Testing

Standard Reference toxicant (SRT) tests provide function as a lab-wide demonstration of consistency in method performance and also are used to assess sensitivity of the lineages of test organisms used in the toxicity testing studies. Sensitivity of organisms is routinely evaluated using in-house reference toxicants. Test organism responses (LC50 for acute tests; IC25 for chronic tests) are compared to specified tolerances using control charts to plot the most recent 20 values. While many organisms used for toxicity testing are purchased from reliable suppliers, any fresh or saltwater test organisms cultured in our laboratory are maintained under the recommended environmental conditions and monitored daily by laboratory personnel.

Control limits for standard reference toxicant tests are calculated in terms of multiple standard deviations from the mean value (e.g. mean LC50 or IC25). Control limits are set at ± 2 standard deviations. Limits are established after a minimum of five data points depending on the analysis. Once established, the control limits are updated whenever more data points are generated, and the control chart is maintained using the most recent 20 values.

These control limits, once calculated, are used to monitor the quality of test organisms and laboratory techniques. When analysis of a standard reference toxicant is completed, the quality control data is reviewed and evaluated against these limits (most recent 20 values).

The following frequency is suggested for SRT testing:

- *Americamysis bahia* (commercially supplied): acute SRT concurrent with client tests or quarterly; modified acute and chronic SRT concurrent with client tests.

- *Arbacia punctulata* (in-house cultures): chronic SRT concurrent with client testing or monthly.
- *Ceriodaphnia dubia* (in-house cultures): modified acute and chronic SRT monthly;
- *Daphnia pulex* (commercially supplied): concurrent with client tests;
- *Daphnia magna* (commercially supplied): concurrent with client tests;
- *Pimephales promelas* (commercially supplied): modified acute and chronic SRT monthly;
- *Menidia beryllina* (commercially supplied): modified acute and chronic SRT concurrent with client tests;
- Other species: concurrent with client tests.

SRT solutions should be logged in and tested following the method protocol for each species and test description. Instructions for preparation of reference toxicants are provided on laboratory bench sheets associated with each test.

SRT test data are entered into the Comprehensive Environmental Toxicity Information System (CETIS) statistical program and the appropriate acute or chronic values generated. The value is entered into the cumulative control chart specific for the species and reference toxicant. Acceptance is judged on the criteria of control response and control limits. If the value falls outside the control limits, the reference toxicant preparation procedures should be re-evaluated. If an error in preparation is detected, the test should be repeated as soon as practical. If no technical errors are detected, the sensitivity of the organisms may be beyond the bounds of acceptability and related client-based toxicity tests may require qualification or reanalysis with new samples. Statistically, one in twenty tests may, by chance alone, fall outside of the control chart boundaries without being viewed as an anomaly if no known contributing factors affected the outcome.

9.9 US EPA DMR QA WET

The annual EPA DMR QA WET proficiency testing program provides a lab-wide demonstration of proficiency in method performance. As part of our Quality System, Aquatec participates in the annual US EPA DMR WET program. This program consists of unknown samples ("simulated effluents") for aquatic toxicity testing. This program provides a measure of accuracy, because results are comparative on a nation-wide scale.

Unknown reference standards are obtained from a commercial supplier (currently ERA) and tested (acute and chronic toxicity tests) in our laboratory. Annual PT standards (DMR QA) must be logged in and handled in the same manner as routine environmental samples using the same personnel, appropriate methods, and equipment. Once the toxicity tests are completed, the results of each test (acute and chronic values) are submitted to ERA within the time frame specified for the program.

When the final DMR graded report is received, it is reviewed by the Director and Laboratory Manager. If all of Aquatec's reported results are graded as "Acceptable" no follow up review of the testing is required and the report is then submitted to clients requesting the results of the study. If any reported values are outside of the acceptance limits Aquatec will review the submitted test data and develop a possible justification for the out of boundary result(s). Following this review, a retest may be performed under the "quick response" testing protocol provided by ERA. The results of the retest will then be supplied to clients requiring the report. The final report and original data associated with each toxicity test are archived in a project folder for each year the study is completed and records are stored for a minimum of five years. These documents are available for review by the Director/QAO, Deputy QAO, and authorized NELAC accreditation personnel.

9.10 Toxicity Test Control Responses and Environmental Conditions

In accordance with toxicity testing methods, each test concentration and control samples are analyzed in replicate. A control sample consisting of the dilution water (for effluent tests) or control sediment (for sediment tests) is included with each concurrent test or group of tests. If the response of the control test organisms is outside control limits for a specific test method, the test conditions are scrutinized for out-of-control situations (e.g. environmental conditions out of specified ranges). The response of the control test organisms and the test conditions are reported with each toxicity test. The interpretation of the response and test conditions may influence the final report. If the test is judged to be unacceptable (by the Laboratory Manager and/or Director) due to organism response and/or laboratory conditions, the test results will be rejected and Aquatec will request a new sample for analysis or retest the same sample depending on sample holding time limits.

Very often our clients provide a receiving water sample to be used as dilution water for effluent toxicity tests. Since Aquatec does not have any control over the quality of receiving water samples supplied to us, when a receiving water control (used as dilution water and as the primary control) fails to meet acceptance criteria, Aquatec will notify

the client and suggest a retest. It then becomes the responsibility of the client to decide whether to schedule a retest.

Aquatec has the appropriate equipment (temperature-controlled spaces and temperature monitoring devices; water quality instrumentation, etc.) to maintain test conditions within the ranges required by the protocols. Test temperature is measured at least once per 24-h period for the duration of the test. When environmental conditions for a toxicity test are outside the range suggested by test protocols, the results of the test should be reported with appropriate qualifiers describing the out-of-range condition. The acceptability of the test will be dependent on the degree of departure from prescribed conditions and the objectives of the test. In some cases, the Laboratory Manager and the Director will review the conditions to determine acceptability. In other cases, appropriate regulatory agencies may be contacted to provide additional guidance regarding test acceptability.

Glass or plastic covers are used to separate on-going tests from cultures or organisms that are being held in proximity.

9.11 Organism Identification

Aquatec cultures some organisms and purchases several others from established and reliable commercial suppliers. The Laboratory Manager and analysts are trained, through experience, to recognize the species commonly used for testing. Purchased organisms are shipped with paperwork provided by the supplier. This paper record includes the organism identity and culturing conditions of the organism. Species cultured at Aquatec (e.g. *Ceriodaphnia dubia and Arbacia punctulata*) are identified annually.

9.12 Lighting Cycles

Light intensity shall be maintained as specified in the method SOPs or manuals. Measurement and documentation of light intensity should be performed at least quarterly. Photoperiods should also be maintained as specified in the method SOPs or manuals and be observed for proper function on a quarterly basis.

9.13 Toxicity Test Methodology

The methods used for toxicity tests are based upon protocols outlined in US EPA, ASTM, or US Army Corps of Engineers publications. In some instances, these

protocols may be modified by State-specific or Project-specific requirements. Minimum test chamber size and test solution volumes, light intensity, and photoperiod, and required environmental conditions are specified in method-specific protocols and SOPs. Our internal SOPs are used to define the general procedures, conditions, and acceptance criteria used for the tests performed at Aquatec. NELAC-format SOPs describe the toxicity tests performed within the parameters of NELAC Accreditation. Also, reports submitted to our clients contain a detailed summary of the method or protocol followed for that particular test. Any deviations from the reported method are noted as qualifiers within the report (See Section 7.4.1 for additional information regarding reporting of toxicity testing results.).

9.14 Standard Operating Procedures

Standard Operating Procedures (SOP's) contain the basic procedures and practices the laboratory uses to complete requested methods or analyses. These procedures provide a basis for training new employees and for providing consistency in the performance of the procedure including quality control standards and ranges of acceptability for a particular method. SOPs may contain proprietary information and as such are controlled documents. In the case of any new procedures that may be developed or obtained from published protocols, documented demonstration of capability (e.g. ability to meet control standards,) must be attained before the procedure is finalized or instituted as a formal SOP. Active SOPs are stored in a binder with a Table of Contents listing the available SOPs. The SOP binder for Environmental Toxicology is located in the Toxicology Laboratory.

The SOP provides information on the preparation of standards or reagent solutions, instrumental functions and operation, and analytical procedures (test methods), and quality control criteria, etc. Nearly every operational procedure in the laboratory should have a supporting SOP however, in some cases manufacturers operational manuals may be used as the operational guideline. Simple "plug-in" apparatus (e.g. air pumps, water pumps, fans, etc.) does not require a formal SOP.

SOP's often reflect standardized or published methods and protocols (e.g. Standard Methods, instrument operation instructions, or EPA-600 methods). While SOPs will carry the basic information required to accomplish a specific procedure, the SOP may not contain all of the information presented in the published document. The function of the SOP at Aquatec is to provide the analyst with the essential information required to be proficient with that procedure. Also, some Aquatec SOPs are written to describe an

"in-house" procedure that may have been developed here, so that methods developed in-house will be documented and repeatable.

As an important element of our Quality System, our SOPs incorporate a signature page for evidence of reading, understanding, and agreement to follow by each analyst who will be involved in performing the procedure. The signature page associated with each SOP contains the printed name, signature, and date read by the analyst. Review and signing by the Laboratory Manager or the Director constitutes formal approval of the SOP.

SOPs are controlled internal documents. This means that they are not for general distribution outside of the laboratory. Appropriate regulatory authorities are allowed to view SOPs, with the Director's approval. If a client requests a copy of an SOP, an electronic or paper copy may be made available, with Director or Laboratory Manager approval.

Method-specific SOPs for NELAC-accreditation should be formatted following the guidance of the NELAC standards (NELAC 2005 Standards, Section 5.5.4.1.2 or updates of the Standard). The following elements are to be incorporated into SOPs pertaining to NELAC-accredited methods:

1. identification of test method
2. detection limit;
3. scope and application;
4. summary of test method;
5. definitions;
6. interferences;
7. safety;
8. equipment and supplies;
9. reagents and standards;
10. sample collection, preservation, shipment, and storage;
11. quality control;
12. calibration and standardization;
13. procedure;
14. calculations;
15. method performance;
16. pollution prevention;
17. data assessment and acceptance criteria for quality control measures;
18. corrective actions for out-of-control data;

19. contingencies for handling out-of-control or unacceptable data;
20. waste management;
21. references;
22. tables, diagrams, flowcharts, and validation data.

Some of the above-listed elements may not apply to a particular method SOP. When this occurs, the words "Not applicable" may be written for that element or section.

Some SOPs are designed as concise instructions (e.g. glassware cleaning and instrument operation) for laboratory analysts. These SOPs do not require all of the above-listed elements that a NELAC-format SOP requires, but should be written in a similar format. In cases where a formal SOP is not in place, manufacturers operation and maintenance manuals are an acceptable substitute.

NELAC method SOP's are reviewed on an annual basis as part of the annual Quality Systems review. Due to the large volume of SOPs carried by Aquatec, it is not practical to do SOP review in a short time-frame, therefore, other SOPs are reviewed and updated as needed to incorporate corrections or method updates. Whenever an SOP is formally revised, a new revision number will be assigned and that version will be stored electronically in a "Final SOPs" folder. Also, paper copies of the most recent version of SOPs will be stored in a notebook in the laboratories – available to all laboratory analysts. The prior electronic version will be archived electronically in an "Expired SOPs" folder and at least one paper copy of the prior version will be archived in a "Expired SOPs" hard copy folder. Any SOPs in the developmental phase will be stored electronically in the "Draft SOPs" folder until it is finalized and then moved into the "Final SOPs" folder. NELAC method or SOPs will be stored electronically in the NELAC SOP electronic folder.

If a minor amendment or clarification is needed for a particular SOP, the amendment may be accomplished through a hand-written annotation on the lab copy of the SOP. Analysts will be required to read and initial the annotation(s) to acknowledge the revision. During the SOP review cycle, this annotation (if appropriate) should be incorporated into the revised final SOP and the revision number (next higher number) should be updated.

10.0 AUDITS AND QUALITY SYSTEM REVIEWS

10.1 Internal Laboratory Audits

NELAC standards require a documented annual internal audit. Internal technical audits and annual Quality System review will be administered by the Director/Quality Assurance Officer with the assistance of Laboratory Managers and the Deputy QA Officer. The audit will involve a "Check List" format, similar to that used for external audits by NELAC auditors. The internal audit must address all elements of the Quality System including environmental testing activities conducted under NELAC accreditation.

The internal audit program will focus on the following areas:

- Maintenance of SOPs in company and NELAC-acceptable format.
- Maintenance of training records.
- Maintenance of notebooks or data packages for each project.
- Maintenance of instrument maintenance and calibration records.
- Evaluation of reagent labeling and tracking.
- Evaluation of standards control records (e.g., laboratory water monitoring).
- Evaluation of sample handling procedures.
- Evaluation of data handling and storage procedures.
- Evaluation of representative specific test methods from sample receipt through reporting.

The findings of internal audits will be formally documented. The Laboratory Manager will have the responsibility for resolving points at issue or for effecting necessary changes to the laboratory's practices in a timely manner.

The target time frame for addressing corrective actions resulting from the internal audit is one month from the completion of the internal audit report. Any follow up audit activities should be documented to record the implementation and effectiveness of any corrective actions.

10.2 Internal Data Audits

The Director/QAO or Deputy QAO will audit or review a representative report and associated data package at least quarterly. This will involve reviewing a current report/data package or one that has been or will be submitted to a client. If an error is

detected that affects the reported outcome of the analysis (for a report already submitted), the client will be notified immediately and a revised report will be submitted to the client. A written narrative will accompany the corrected report notifying the client of the error with linkage to the original report provided. The results of the data audit will be documented.

If an audit indicates that a client's analytical results are questionable, the client will be notified and a decision will be made as to whether a report revision or possibly re-analysis of samples is required. Aquatec will then work with the client and make every attempt to resolve any issue(s) needing attention.

If substantive errors are detected by an internal data audit, the Director/Quality Assurance Officer will verify that corrective action has been implemented. Observations made during this follow up will be made available to the appropriate representatives of the regulatory agency upon request.

10.3 Audits by Regulatory Agencies or Accrediting Authorities

As a participant in state and federal certification programs and the New Hampshire Environmental Laboratory Accreditation Program (NH ELAP), Aquatec may be periodically audited by representatives of these agencies. Audits typically focus on laboratory conformance to the specific program protocols for which the lab is seeking certification or accreditation. Auditors are likely to review sample handling and tracking documentation, traceability (of chemicals, standards, and reagents), analytical methodologies, analytical supportive documentation, and final reports. Audit findings from regulatory agencies are formally documented.

State of New Hampshire Department of Environmental Services Environmental Laboratory Accreditation Program: Aquatec submits required documents for annual renewal of NELAC accreditation. The cycle of NH ELAP (NELAC) laboratory on-site audits is typically every two years. The Quality System is reviewed to address any deficiencies resulting from accreditation audits.

10.4 Client Audits and Clarifications of Statements of Work

Aquatec provides opportunities for clients or potential clients to visit our facility, tour our general operations, and to discuss and clarify any proposed or upcoming work with that client. Care is taken not to compromise the confidentiality of existing clients and projects. For example, if a visiting prospective client requests to see an example report,

a copy of an original report with any reference to other client's names or means of identity should be obscured.

Aquatec also maintains open lines of communication with clients, via phone and e-mail, such that any aspects or misunderstandings or performance issues can be readily discussed and resolved.

10.5 Response to External Audits

Aquatec will prepare a formal written response to audit findings from regulatory or accrediting authorities and will develop a plan to address any required corrective actions. The written response will address each item presented in the audit report and will be submitted to the auditing authority.

If a recommendation is related to revising particular documents to meet the standards required by the audit, then the revisions will be made and a copy of the revised document(s) will be forwarded to the auditor, if required.

If a recommendation is related to a laboratory procedure (for example, error correction), then the recommendation will be communicated to the laboratory personnel informing them of the correct procedure and a record of this communication will be submitted to the appropriate representatives of the regulatory agency. In conjunction with this, the QAPP or SOPs may be annotated to incorporate the required corrections or updates. If hand-written updates are incorporated into QA documents, the annotated updates must be read, initialed, and dated by laboratory personnel. These annotations will be incorporated into future revisions of the QA documents (QAPP and SOPs). A copy of the annotations or the revised QAPP or SOPs will be made available to the auditor upon request.

10.6 Management Review of Quality System

On an annual basis, management (Director/QAO and Lab Managers) will conduct a review of the Quality System as required by NELAC Standards. The purpose of this review is to ensure the suitability and effectiveness of the program, to ensure that certification standards are met, and to provide opportunity for improvements. The review includes the following topics:

- Follow-up on decisions made in meetings;
- Evaluate reports from audits by clients and regulatory agencies;

- Evaluate reports from internal audits;
- Evaluate results of proficiency studies;
- Evaluate results of water quality monitoring;
- Evaluate corrective actions from the past year and implement;
- Evaluate details of complaints from clients and their resolutions;
- Evaluate training goals and objectives;
- Evaluate staff, facility and equipment resources;
- Evaluate future plans and goals; and,
- Revise the QAPP, Employee Manual, and SOPs, as needed.

The Management Review must address any known evidence of inappropriate actions or vulnerabilities related to data integrity. Any potential issues must be handled in a confidential manner until a follow up evaluation or full investigation occurs. Any finding, corrective action, or disciplinary action(s) resulting from known compromises of data integrity must be documented with records maintained at least five years.

A checklist similar to that presented in Appendix E of this QAPP may be used to facilitate and document the annual Management Review.

11.0 CERTIFICATIONS AND ACCREDITATIONS

Aquatec maintains NELAC accreditation for WET testing through the State of New Hampshire Department of Environmental Services Environmental Laboratory Accreditation Program (NH DES ELAP). A copy of Aquatec's current NELAC certificate and associated parameter list is found in Appendix D.

Aquatec has also been approved by the US Army Corps of Engineers and US EPA, New England Divisions for biological assessments associated with proposed dredging activities within the New England Region.

Certain personnel at Aquatec hold current certification for OSHA CFR 1910.120 HAZWOPER training. OSHA HAZWOPER training may be a requirement for some project-specific activities.

12.0 CORRECTIVE ACTION AND NON-CONFORMING WORK

To the extent possible, results are reported only when quality control measures are acceptable. If a quality control measure is found to be out of acceptance range, and the data are to be reported, all samples associated with the failed quality control measure

shall be reported with the appropriate data qualifiers and evidence of the non-conforming event. A sample retest or retest with new samples may be recommended to the client if QC parameters are outside acceptance ranges or do not conform to agreed requirements of the client.

When deficiencies or "out-of-control" situations exist, the Quality Systems program provides a means for detecting, documenting, and correcting these situations. Samples analyzed during out-of-control situations may be reanalyzed prior to reporting of results or re-sampling and re-testing may be required.

A Corrective Action Report, (Appendix E) is available for use in documenting events. This document may be completed and filed for situations that represent either singular or systematic QA or QC problems. Or, documentation of out-of-control situations may be limited to notations made on Chain-of-Custody forms (e.g. for samples received) or laboratory bench sheets for specific test methods, when appropriate. Samples broken in-transit, missed holding times, suspect standard/reagent lot, non-compliant calibration, or software problems are examples of situations that may require corrective action. Document or notations associated with corrective actions are stored in project files or folders. The culmination of corrective action reports or notes will be used to assist the project director in preparing a narrative or qualifiers to be included in the client report.

There are several categories of "out-of-control" situations that may occur in the laboratory during analysis and result in the need for corrective action.

12.1 Receiving-level Corrective Action

Discrepancies between the Chain-of-Custody documentation and sample labels may become evident as samples are logged in. Examples of other possible discrepancies include: broken sample containers; inappropriate containers; mislabeled containers; improper preservation; improper temperature; or, extended holding times. Any discrepancies such of these must be noted either on the Chain-of-Custody form or on log-in work sheets or as notes added to the project file. Corrective action may involve contacting the client to potentially clarify or resolve the problems. Analysts should notify the Laboratory Manager so that appropriate decisions can be made regarding continuing, delaying, or cancelling analysis of samples (with client approval). Information or summaries of communications regarding corrective actions must be documented and included in the project file. Appropriate qualifiers should be applied to results from procedures that required corrective actions.

12.2 Bench-level Corrective Action

While some events in the laboratory require corrective action, they may not be viewed as "out of control" events. An example of this would be where a miscount of organisms has been made in a toxicity test replicate or an error in recording water quality data. The requirement for correcting test data is described in Section 13 of this QAPP.

An event potentially requiring minor corrective action for an unexpected non-conforming event requires that the analyst, as a minimum, write a notation on the bench sheets or attached paperwork describing the event in sufficient detail such that the event can be reconstructed for reports. The analyst must also notify the Laboratory Manager that a "lab error" has occurred. An example of this, in the toxicity laboratory, would be an error resulting from accidentally spilling a test replicate. In this example, the Manager and/or Director must make a decision as to whether the toxicity test should continue (with reduced replication at one treatment level), or whether a retest should be scheduled. In some cases, the final decision as to the impact of the event may not occur until a formal review of the data has been completed however a description of any known laboratory errors resulting in deviation from test protocol or other non-conforming events must be described in the QA/QC narrative associated with the report.

Other corrective action procedures are often handled at the bench level. If an analyst finds a non-linear response during calibration of an instrument, then the instrument is recalibrated before sample analysis commences. The instrument will also be recalibrated if reference or internal standard results are outside of specified tolerances. On rare occasions, paperwork associated with a commercial reference standard may be incorrect. If there is reason to suspect that reference values do not concur with expected results (following standard procedures), the supplier of the product should be contacted to investigate the potential of incorrect information provided with the product. Problems are often corrected by a careful examination of the preparation or calibration procedure or instrument sensitivity. If a problem persists, it is brought to the attention of Lab Manager or Director. If an analyst is aware of an unusual event that could affect the test result or data quality (e.g. spilled test replicate, unusual mortality) during an analysis the analyst must document the event and bring it to the attention of the Laboratory Manager and/or the Director.

12.3 Management-level Corrective Action

If resolution at the bench level was not achieved or a deficiency is detected after the data has left the bench level, corrective action becomes the responsibility of the

Department Manager or Director. A decision to reanalyze the sample, stop the analysis, or report the results (with appropriate qualifiers) is made depending on the circumstances of the sample and analysis. Documentation procedures for sample re-analysis or reasons for stopping the analysis are initiated at this point if necessary. A corrective action report should describe the circumstances, decisions, resolution, and follow-up aspects of the corrective action. A corrective action report (Appendix E) or copies of phone logs and communications should be filed in the project file and may be archived electronically in QA/Toxicology QA/Corrective Action Reports.

12.4 Statistical Events

An out of control statistical event is defined as data exceeding control limits, unacceptable trends detected in the charts, or unusual changes in the instrument detection limits. If these situations arise, they are brought to the attention of the Department Managers or Director who will initiate the appropriate corrective action. Corrective action may take the form of instrument maintenance, recalibration, or re-analysis of the sample.

12.5 Client Complaints and Data Validation Feedback

If a client or data reviewer has a question or finds a deficiency concerning the data submittal, the Laboratory Manager or the Director, is responsible for communicating and implementing the corrective action. The analytical results and supportive documentation will be re-evaluated. Should a reanalysis be necessary, it may be initiated if the sample is still available and within prescribed holding times. Since microbiology samples and effluent samples have short holding times, re-analysis of stored samples might not be practical and re-sampling might be necessary. If revisions to the report are necessary, the corrections are integrated into a revised report that is submitted to the client.

Hard copies and revised electronic deliverables (where applicable) will be re-submitted to the client when appropriate. In some instances, clients request that additional sample handling information, recalculations, or qualitative judgments be provided. In this case, resubmission of the data may not be necessary unless a discrepancy or error is found with the original data or report.

Customer complaints will be documented and the report filed in the complaints file within the administration office or in project files. This report will have at a minimum: Client contact information; 2) Type(s) of analyses; 3) Complaint; 4) Documented

Resolution(s); 5) Person handling the complaint. Customer problems that persist in the laboratory are to be communicated to the Director and where appropriate, corrective action will be initiated. A form such as that shown in Appendix E can be used to document client complaints. Completed forms should be stored in the project folder and also in QA/Toxicology QA/Client complaints.

12.6 Cause Analysis

When nonconforming work or departures from policies or procedures have been identified, the procedure for corrective action will start with an investigation to determine the cause. Corrective actions will be to a degree appropriate to the magnitude and risk of the problem. Action(s) most likely to eliminate the problem and prevent recurrence should be implemented. Use of the Corrective Action Report (Appendix E) will provide a format for identifying, describing, and resolving corrective action needs.

Resolution of departures may require documented improvements to Quality System documents (QAPP, SOPs, and Employee Manual) or project-specific work plans to address any persistent or misunderstood aspects of the required work. If problems with nonconformance or departures from NELAC Standards or protocols persist, laboratory or personal audits may be required to re-establish conformance and if required, issue warnings to applicable employees.

13.0 LABORATORY DOCUMENTATION

Employees generating data are required to be included in the log of names, initials, and signatures associated with this version of the QAPP. The initials (or variations) of the QAPP should match the initials (or variations) of individuals documenting observations. Handwritten data and observations must be recorded directly and promptly at the time of observation. Employees recording original data are required to provide initials and date associated with the data when the data are generated. Recording the time of observations is optional unless there is an indication on the bench sheet that the time should also be recorded. If time is requested, the convention is to write the time when the procedure is completed (e.g. time a complete test is started or ended, time of daily survival counts, renewal, etc.). Our bench sheets are designed to query for this sign-off information to be included along with the recorded data or observations. The ruling standard is that documentation can be clearly traced to an analyst and date and also time in many circumstances. There are instances when sets of data are common to more than one test. Data transcriptions to complete an associated bench sheet are

discouraged. A note should be on the unused data sheet, making reference to the associated and common data.

Workbooks, bench sheets, instrument logbooks, and instrument printouts, are used to trace the history of samples through the analytical process and to document and relate important aspects of the work, including the associated quality controls. All logbooks, bench sheets, instrument logs, and instrument and statistical analysis printouts are part of the record of the laboratory work. Completed workbooks and/or bench-sheets, are submitted as part of the data package for review. These are stored, with the final report, in the appropriate project file. Active instrument logbooks and water and reagent preparation logs are stored in the laboratory. At the end of a calendar year the logbooks are filed with project files for that year and archived for at least five years.

13.1 Data Recording Errors

Errors in entry are to be crossed out in indelible ink with a single stroke and a corrected entry made. White-out, obliteration, or write-over of recorded data are not permitted. All corrections are to be initialed and dated by the individual making the correction. In some cases (e.g. data used to generate end points for analysis), justification for the data change should be documented. Provide a clear and concise justification for making a change or correction. Pages inserted into permanent logbooks are to be stapled or taped to a clean, bound page. The analyst's initials are to be recorded in such a manner that the initials overlap the inserted page and the bound page. Pages of bench sheets or logbooks which are not completed (as part of normal record keeping) should be completed by lining out unused portions.

14.0 DOCUMENT CONTROL

14.1 Quality System Documents

Security of internal documents is necessary to ensure that confidential information is not distributed and to make sure that active copies of a given document are from the latest applicable revision. Internal controlled documents have a header placed in the upper right hand corner of each page. This header provides enough information to unambiguously identify each page as part of a single compiled document. At a minimum, the following information will be supplied:

Document Name:
Revision Number:

Revision Date:
Page ___ of ___

The Director/QAO and Department Managers control the following documents for Aquatec Biological Sciences, Inc.:

- Quality Assurance Program Plan
- Standard Operating Procedures
- Employee Manual
- Chemical Hygiene Plan

These documents are made available to authorized accrediting agencies, upon request and following Director approval.

Periodically clients may request access to or copies of certain internal documents (SOPs or QAPP) for incorporation into project-specific QAPPs or Sampling and Analysis Plans (SAPs). With approval from the Director, these documents may be provided on a case-by-case basis. Internal documents provided to clients should be marked with the following header information "Controlled document - Do not duplicate."

Revisions of Quality Systems documents may be in the form of hand annotations followed by the annotation being read, initialed, and dated by laboratory personnel. Approved hand annotations should be formally incorporated into the next formal revision of the document. Prior versions of internal documents are stored electronically on Aquatec's secure server and expired paper copies are stored at least five years in administrative files.

14.2 Client Documents and Reports

Reports and data sent to clients are to be viewed as confidential information. These documents do not need to be stamped as "confidential" unless specifically requested by the client. If a client requests preliminary information regarding a specific project (e.g. by phone or e-mail) there must be certainty that information is provided only to the authorized contact person.

If changes or revisions to a data report are submitted to a client to correct for errors or omissions, the revised document must be clearly marked as "revised" or "Supplement to Test Report #", or equivalent wording. If a complete new report is issued, the new test report should be uniquely identified and contain reference to the original. A cover letter

should accompany the revised document that provides an explanation of changes and reference to the original document. Copies of original and revised documents must be stored in project folders and project electronic files.

15.0 STORAGE OF RECORDS

All levels of data and associated paperwork and reports generated at Aquatec, documents are stored for a minimum of five years in metal file cabinets identified by year. After five years, documents may be discarded through destruction (e.g. shredding). Electronic copies of reports (PDF) are also stored within project folders on our secure server. Documents may be stored for longer than five years (e.g. for legal or informational purposes) as long as the documents remain whole and original identity and purpose of the documents (e.g. project reports, proposals) is clear.

Data associated with laboratory operations including internal audits, management reviews, calibration records, test organism records, laboratory water analysis data, DMR reports, reference toxicant data, etc. are stored for a minimum of five (5) years in the appropriate file folders for that year.

In the event of transfer of ownership or other event resulting in the termination of Aquatec Biological Sciences, Inc. as a business, records will be maintained or transferred according to clients instructions or are archived in a secure and announced location for a minimum of five (5) years from the date of the analysis or project work.

16.0 SAMPLE DISPOSAL

Method-specific SOPs outline sample disposal, pollution prevention, and waste management procedures followed at Aquatec. Appendix B of this QAPP provides additional guidance regarding storage and disposal of samples.

Unused sample portions and extracts are stored appropriately (usually refrigerated or frozen) for the amount of time specified by contractual obligations and project specifications after the submission of the data package. After their storage period expires, the samples may be removed from the refrigerator/freezer and stored in a secured storage area. Unused sample portions removed from the refrigerators may be drummed, packed, and disposed of as either non-regulated or hazardous waste depending on the results of the analyses or instructions from clients.

Aquatec has a non-hazardous and hazardous waste storage area containing 55-gallon drums for sorting and packing lab waste. The waste is packed according to specifications outlined in Federal Regulations and Department of Transportation regulations. Periodically, the waste is transported by an approved waste hauler, to an appropriate disposal/treatment facility.

The following general guide-lines apply to sample disposal:

NPDES effluents and receiving waters: Unused residual aqueous samples may be discarded after one week following test completion (unless otherwise directed). Discarded water from toxicity tests or cultures may include live organisms. Any of these solutions must be treated with disinfection (bleach) prior to discarding to normal drainage. Samples that are shown to be potentially highly toxic (e.g. LC50 less than 10%) should be transferred to the aqueous waste drum for disposal by a waste disposal firm. Consult with the Laboratory Manager regarding specific cases of demonstrated high toxicity.

Sediments and Soils: Client-generated sediments and soils may be discarded 30 days after the report has been submitted to the client unless a client requests an extended storage time for these samples. Residual sediments and soils are discarded in the solid waste drums for disposal by a waste disposal firm. Control sediments collected or prepared by Aquatec may be discarded with normal trash collection.

Reference toxicants: Whenever possible, Aquatec uses reference toxicants (sodium chloride and potassium chloride) that are not harmful to humans in routine use and can be disposed of through normal drainage. Copper sulfate is used as the reference toxicant for the sea urchin, *Arbacia punctulata*, fertilization and embryo development tests. Stock solutions made up with this material are discarded to the aqueous waste drum.

Formalin: Small volumes of formalin waste (e.g. less than 1L) are transferred to a drum or secure container specifically labeled as "formalin waste" for disposal by a waste disposal firm.

Acids and bases: These solutions are neutralized by dilution before release to the normal sink drainage.

Acetone waste: Waste acetone in transferred to a specific "Acetone Waste" drum, for disposal by a waste disposal firm.

Microbiology waste: Unused samples are discarded after disinfection (with bleach) to normal drainage. Used or expired media and liquid or solid waste from analyses (media, petri dishes and contents) are autoclaved before disposal to regular waste.

Limnology: solutions and waste from ammonia analysis generally are of very small volumes (e.g. 5-20 mL) of pore water or aqueous solutions. These samples may be discarded with normal laboratory waste (sink drain) following disinfection with bleach. Waste chemical reagents used in the Alpkem instrument (e.g. phenol, NaNP) are transferred to the aqueous waste drum for disposal by a waste disposal firm.

DOCUMENT SIGNATURE PAGE

DOCUMENT NAME: Quality Assurance Program Plan

Printed Name	I have read and I understand and I agree, to the best of my ability, to follow the procedures outlined in this Quality Assurance Program Plan. Signature	Initials	Date
Philip Downey	Philip C Downey	PCD	3/28/12
John Williams		JW	3/28/12
Jennifer Garrison	Jennifer Garrison	JG	3-29-12
Kaitlyn Koch	Kaitlyn Koch	KK	4/1/12
Stuart Randall			
Karen Downey	Karen Downey	KD	4/2/12

APPENDIX A:
RESUMES

Resumes

Resumes for specific personnel are included in the following pages. Additional resumes are available upon request.

Philip C. Downey, Ph.D.
Director
Quality Assurance Officer
Manager, Ecology and Limnology

Karen Downey
Administration

John W. Williams B.S.
Manager, Environmental Toxicology

Jennifer Garrison, B.S.
Deputy Quality Assurance Officer
Macro-invertebrate sorting / taxonomy / field sampling
Environmental toxicology support
Limnology analyses
Chlorophyll a / Phytoplankton

Kaitlyn Koch, B.S.
Environmental Toxicology support
Macro-invertebrate sorting / field sampling /Taxonomy
Icthyoplankton sorting / field sampling /Taxonomy
Data entry and analysis

Stuart Randall, B.S.
Zooplankton taxonomy
Field support

Philip C. Downey
Primary Responsibility: Director, 1996 to Present
Duration of employment: 1996—current

Education/Field of Specialization
Ph.D. in Forestry Wildlife and Range Sciences, 1982 University of Idaho, Moscow
Fisheries Resources
Master of Science in Fisheries, 1978 Louisiana State University, Baton Rouge
Bachelor of Science in Natural Resources, 1975 University of Michigan, Ann Arbor/Fisheries
Bachelor of Science in Biology, 1975 Marietta College, Marietta, Ohio

Affiliations, Registrations, and Specialized Training
2004-present: Certified Fisheries Professional (American Fisheries Society)
2011: Hazardous Materials Incident Response Operations (165.5), renewed annually
2010: OSHA 1910.120 Training (annual refresher)
1991: SCUBA, NAUI Advanced Certification
1991: Dale Carnegie-Effective Speaking and Human Relations
1984: Habitat Evaluation Procedures (HEP), Certified
1985: Hydro-acoustic Assessment Techniques, Biosonics, Inc.

Professional Associations
2010-2012: American Fisheries Society NE Division, President/President-elect
1995-1997: American Fisheries Society NE Division, Secretary Treasurer
1991-1992: American Fisheries Society Atlantic International Chapter, President
1974-present: American Fisheries Society, Member
1985-present: Vermont Subcommittee on Endangered Fishes
2006-2008: Vermont Subcommittee on Endangered Fishes, Chairman
1994-2011: Society of Environmental Toxicology and Chemistry (SETAC)
1975: Phi Sigma Biology Honor Society

Representative Recent Project Experience (2003-2012)
2012: Aberdeen Proving Grounds, MD. A&E firm: *Lumbriculus variegatus* sediment bioaccumulation.
2011: Confidential: *Chironomus tentans*, *Hyalella azteca*, and earthworm, *Eisenia fetida* chronic toxicity assessment
2011-present: Great Lakes Fish Monitoring and Surveillance Program. A&E Firm/USEPA: Aging, condition, electronic tag removal, preparation of top predator fish for monitoring of bioaccumulation of emerging contaminants & fish health.
2011-2012: Allen Harbor, MA dredge sediment assessment. Town of Harwich/US Army Corps of Engineers Analyze bioaccumulation body burdens and prepare Risk Assessment reports .
2010: Ashtabula Harbor, Ohio. TestAmerica/US Army Corps of Engineers. *Lumbriculus variegatus* bioaccumulation
2009-2011: Ichthyoplankton Assessment, New London/Groton, CT. A&E Firm: Collect and identify ichthyoplankton with a focus on winter flounder.
2007-2009: Connecticut River Fisheries and Macroinvertebrate Assessment, Hartford, CT. A&E Firm: Comprehensive ecological investigation of fish community structure, adult fish movement, ichthyoplankton distribution, and macroinvertebrate community evaluation.
2007-present: Glowegee Creek Annual Fish Community Assessment, Galway, NY. USN: Conduct an annual electrofishing study of the fish community to monitor spatial and temporal trends.
2005-2009: Freshwater and marine macroinvertebrate Taxonomic Studies, Various Locations.
2002-2006: Allen Brook Water Quality Evaluation, Winooski, VT: Fisheries and macroinvertebrate studies to evaluate whether water quality in the target reach had improved to remove it from Vermont's list of impaired waters.
2005-200: Evaluation of dredge sediments for disposal. A&E Firms and USACE: Coauthored reports on sediment toxicological and bioaccumulation laboratory results associated with harbor dredging in the New England Region.
2003-2005: Merrimack River Watershed Water Quality Study: Microbiological support with on-site processing over 1000 microbiological analyses during diurnal studies of spate conditions.
2008-2009: Onandoga Lake Fish Tissue Body Burden: Prepared of fish for evaluation of the use of non-lethal techniques to obtain tissues for contaminant body burden analyses.

Philip C. Downey, Ph.D
Aquatec Biological Sciences, Inc.
273 Commerce Street, Williston, VT 05454
Phone: (802) 860-1638 e-mail: pdowney@aquatecb.com

Resume update:
March 2012

John Williams
Primary Responsibility: Manager, Environmental Toxicology Laboratories
Additional Responsibilities:
Ecological Field sampling support, Laboratory Quality Assurance (NELAC)
Duration of employment: 1996—current

Education /Field of Specialization
B.S., 1968 University of Massachusetts, Amherst, Biology/Fisheries
Graduate-level courses: Harvard University Extension Program, Boston University

Affiliations, Registrations, and Specialized Training
2009-2010: Executive Board of Directors, North Atlantic Chapter of SETAC
2008-2009: President, North Atlantic Chapter of SETAC
2007-2010: Board of Directors, North Atlantic Chapter of SETAC
2010: OSHA 1910.120 Training
1996-present: Member, Society of Environmental Toxicology and Chemistry
1975 NAUI Certified SCUBA Instructor

Presentations and Publications
2010 Platform Presentation: *A Simple Device for Controlling pH in Aqueous Solutions for Toxicity Evaluations.* North Atlantic Chapter of SETAC 16th Annual Meeting, Narragansett, RI
2007 Short Course Co-Instructor: *Sediment Toxicity Testing, Methods to Achieve Strong Data Sets and Interpret Results*, North Atlantic Chapter of SETAC 13th Annual Meeting, Roger Williams University, Bristol, RI
2006 Short Course Co-Instructor: *Innovative Sampling Techniques for Surface Water, Sediment, and Pore Water*, 12th Annual Meeting North Atlantic Chapter of SETAC, Portland, ME
2004 Platform Presentation: *On-Site Toxicity Assessment Using the Sea Urchin, Arbacia punctulata*, North Atlantic Chapter of SETAC 10th Annual Meeting, Portsmouth, RI

Representative Recent Project Experience (2006-2012)
NPDES Compliance Testing (Whole Effluent Toxicity)
1996-present: Freshwater and marine WET for municipal and industrial clients throughout the northeast and Puerto Rico.

Freshwater Sediment and Soil Toxicity and Bioaccumulation
2012: Aberdeen Proving Grounds, MD. A&E firm: *Lumbriculus variegatus* sediment bioaccumulation
2011: Confidential: *Chironomus tentans*, *Hyalella azteca*, and earthworm, *Eisenia fetida* chronic toxicity assessment
2010: Lockheed Martin SERAS Program Red Mud Assessment: *Chironomus tentans*, *Hyalella azteca*, and *Leptocheirus plumulosus* chronic toxicity
2010: Ashtabula Harbor, Ohio. TestAmerica/US Army Corps of Engineers. *Lumbriculus variegatus* bioaccumulation
2010: RCRA Facility in Windsor Locks, CT. A&E firm: *Chironomus tentans* and *Hyalella azteca* chronic toxicity
2010: Cranston, RI Landfill. A&E firm: Pore water and chronic sediment toxicity tests with *Hyalella azteca*

Marine/Estuarine Sediment Toxicity and Bioaccumulation
2011-2012: Allen Harbor, MA dredge sediment assessment. Town of Harwich/US Army Corps of Engineers
Arbacia punctulata, *Menidia beryllina*, and *Americamysis bahia* suspended phase toxicity; *Ampelisca abdita* survival. 28-day bioaccumulation with *Nerieis virens* and *Macoma nasuta.*
2010: Oil dispersant toxicity and dispersant effectiveness study. Summit Scientific
2010: New Haven Harbor Dredged Material Assessement. A&E firm /US Army Corps of Engineers
Arbacia punctulata, *Menidia beryllina*, and *Americamysis bahia* suspended phase toxicity; *Ampelisca abdita* survival. 28-day bioaccumulation with *Nerieis virens* and *Macoma nasuta.*
2009: Pearl Harbor Landfill sediment assessment . TestAmerica
Leptocheirus plumulosus 28-day chronic test and *Nereis virens* 28-day bioaccumulation.
2009: McAllister Point Landfill, A&E firm /US Navy
Pore water toxicity with sea urchin *Arbacia punctulata* fertilization tests, sediment assessments with amphipods *Ampelisca abdita* and *Leptocheirus plumulosus*,
2006: Massachusetts Bay Dredged Material Site Management and Monitoring, USEPA/Alpha
Ampelisca abdita 10-day whole sediment toxicity tests.

John Williams, Aquatec Biological Sciences, Inc.
273 Commerce Street, Williston, VT 05495
Phone: (802) 860-1638
e-mail: jwilliams@aquatecb.com

Resume update: March 2012

Jennifer Garrison

Primary Responsibility:
Environmental Toxicology Laboratory Analyst
Deputy Quality Assurance Officer
Additional Responsibilities:
Ecological Field sampling support
Chlorophyll *a* Analysis
Inorganic chemistry (ammonia)
Microbiology/Microtox
Toxicology laboratory analyst
Duration of employment: 1998—current

Education
B.S., 1998 Johnson State College, Biology

Affiliations and Registrations
2009 OSHA HAZWOPER 40-hour (Title 29 CFR 1910.120) and annual updates

Representative Recent Project Experience (1996-Present)
NPDES Compliance Testing (Whole Effluent Toxicity)
1996-present: Freshwater and marine WET for municipal and industrial clients throughout the northeast and Puerto Rico.

Freshwater Sediment and Soil Toxicity and Bioaccumulation
2012: Aberdeen Proving Grounds, MD. A&E firm: *Lumbriculus variegatus* sediment bioaccumulation
2011: Confidential: *Chironomus tentans*, *Hyalella azteca*, and earthworm, *Eisenia fetida* chronic toxicity assessment
2010: Lockheed Martin SERAS Program Red Mud Assessment: *Chironomus tentans*, *Hyalella azteca*, and *Leptocheirus plumulosus* chronic toxicity
2010: Ashtabula Harbor, Ohio. TestAmerica/US Army Corps of Engineers. *Lumbriculus variegatus* bioaccumulation
2009: Pearl City Hawaii Landfill soil assessment Test America: *Earthworm, Eisenia fetida* 28-day bioaccumulation, .
2009: Site in Milwaukee, WI sediment assessment for ARCADIS 2009. *Hyalella azteca* 28-day survival and growth toxicity tests
2008: Site in DuPue, IL sediment assessment. ARCADIS. *Chironomus tentans* 10-day survival and growth toxicity tests
2007: Holliston, MA Wetlands sediment assessment. Brown & Caldwell: *Hyalella azteca* 42-day survival, growth, and reproduction toxicity tests, *Chironomus tentans* life-cycle toxicity tests
2006: Chanute AFB. URS. Earthworm *Eisenia fetida* soil bioaccumulation assessment
2006: Site in West Brookfield, MA. Menzie-Cura & Associates: *Lumbriculus variegatus* 28-day bioaccumulation

Marine/Estuarine Sediment Toxicity and Bioaccumulation
2011-2012: Allen Harbor, MA dredge sediment assessment. Town of Harwich/US Army Corps of Engineers *Arbacia punctulata*, *Menidia beryllina*, and *Americamysis bahia* suspended phase toxicity; *Ampelisca abdita* survival. 28-day bioaccumulation with *Nerieis virens* and *Macoma nasuta.*
2009: Pearl Harbor Landfill sediment assessment, Test America: *Leptocheirus plumulosus* 28-day survival, growth, and reproduction test and *Nereis virens* 28-day bioaccumulation.
2008: McAllister Point Landfill, Test America / US Navy: Pore-water toxicity with sea urchin *Arbacia punctulata* fertilization tests, sediment assessments with amphipods *Ampelisca abdita* and *Leptocheirus plumulosus*,
2008: Hingham Harbor Dredged Material Assessement , CLE , US Army Corps of Engineers Elutriate tested with *Arbacia punctulata*, *Menidia beryllina*, and *Americamysis bahia*. Whole sediment toxicity was assessed with 10-day *Leptocheirus plumulosus* survival. Bioaccumulation potential was assessed through 28-day exposures with *Nerieis virens* and *Macoma nasuta,*
2006: Massachusetts Bay Dredged Material Disposal Site Management and Monitoring, USEPA /Alpha: *Ampelisca abdita* 10-day whole sediment toxicity tests.
2008: Hingham Harbor Dredged Material Assessement , CLE , US Army Corps of Engineers Elutriate tested with *Arbacia punctulata*, *Menidia beryllina*, and *Americamysis bahia*. Whole sediment toxicity was assessed with 10-day *Leptocheirus plumulosus* survival. Bioaccumulation potential was assessed through 28-day exposures with *Nerieis virens* and *Macoma nasuta.*

Jennifer Garrison, Aquatec Biological Sciences, Inc.
273 Commerce Street, Williston, VT 05495
Phone: (802) 860-1638
e-mail: jgarrison@aquatecb.com

Resume update: March 2012

Kaitlyn Koch

Primary Responsibility: Ecology Field and Laboratory, Database Management
Additional Responsibilities:
Toxicological data entry, statistical analysis
Toxicology Laboratory analyst
Duration of employment: 2005-current

Education
B.S., 2004 Plymouth State University, Environmental Biology

Additional Training :
2009 OSHA HAZWOPER 40-hour (Title 29 CFR 1910.120)

Affiliations and Registrations
American Fisheries Society (2010)

Representative Recent Project Experience (2006-Present)

Macroinvertebrate and Sediment Ecology
Hazardous waste sites. Provided field support for Benthic/Sediment Sample processing. In the laboratory, processed macroinvertebrate (freshwater and marine) samples, slide preparation, data entry and report writing support.

Fisheries and Field Sampling
2011-present: Great Lakes Fish Monitoring and Surveillance Program. A&E Firm/USEPA: Aging, condition, electronic tag removal, preparation of top predator fish for monitoring of bioaccumulation of emerging contaminants & fish health.

2010-2011: Thames River: Participated in the collection of ichthyoplankton – 1/2m tow net. Laboratory sample processing, enumeration and identification support of larval fish and eggs.

2009-2011: Mohawk River, Farmington River and Glowegee Creek: Participated in the collection of fish with an electro-shocking backpack and with an electro-shocking boat. Laboratory sample preparation of frozen filets and preserved fish samples. Supported written reporting, graphs and tables.

2009-2010: Connecticut River and Tributary: Participated in the collection of: fish – boat and backpack electro-shocking, ichthyoplankton – tow nets (1m and 1/2m nets) and macroinvertebrates – dip net collection. This project included sample processing for ichthyoplankton and macroinvertebrate enumeration / identification. Supported written reports, data entry and graphs and tables for reporting purposes.

Taunton River: Participated in the laboratory sample processing, enumeration and identification of larval / juvenile fish. Supported written reports and data entry.

Toxicity Laboratory Support
NPDES Compliance Testing (Whole Effluent Toxicity)
1996-present: Freshwater and marine WET for municipal and industrial clients throughout the northeast and Puerto Rico.
1996-present: Evaluation of Sediments:
Participated in performing whole sediment toxicity tests in support of risk assessments at marine and freshwater sites. The projecs involved testing whole sediment samples with *Hyalella azteca, Chironomus tentans, Ampelisca abdita, and Leptocheirus plumulosus, Menidia beryllina, Americamysis bahia, Lumbriculus variegatus, Neries virens, and Macoma nasuta.*
2011-2012: Allen Harbor, MA dredge sediment assessment. Town of Harwich/US Army Corps of Engineers
Arbacia punctulata, *Menidia beryllina*, and *Americamysis bahia* suspended phase toxicity; *Ampelisca abdita* survival. 28-day bioaccumulation with *Nerieis virens* and *Macoma nasuta.*

Kaitlyn Koch, Aquatec Biological Sciences, Inc.
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Resume update: March 2012

Stuart Randall

Primary Responsibility: Field Sampling Support
Additional Responsibilities:
Toxicology Lab support
Ecology Lab support (zooplankton identification, macro-invertebrate sorting)

Duration of employment: 2004-current

Education
B.S., 2000 Johnson State College, Environmental Biology
A.S., 1986 Data Processing

Additional Training
Affiliations and Registrations
Representative Recent Project Experience (2004-2009)

Ecology Laboratory:
Zooplankton identification.
Support for field sampling, electrofishing, icthyoplankton and macro-invertebrate collection.
Macro-invertebrate sample processing and sorting of specimens.
Fish filet prep of tissue samples for chemistry analysis.

Fisheries and Field Sampling
2011-present: Great Lakes Fish Monitoring and Surveillance Program. A&E Firm/USEPA: Aging, condition, electronic tag removal, preparation of top predator fish for monitoring of bioaccumulation of emerging contaminants & fish health.
2010-2011: Thames River: Participated in the collection of ichthyoplankton – 1/2m tow net.
2009-2011: Mohawk River, Farmington River and Glowegee Creek: Participated in the collection of fish with an electro-shocking backpack and with an electro-shocking boat.
2009-2010: Connecticut River and Tributary: Participated in the collection of: fish – boat and backpack electro-shocking, ichthyoplankton – tow nets (1m and 1/2m nets) and macroinvertebrates – dip net collection. .

Sediment Toxicology Support:
2008-present: Participated in performing whole sediment toxicity tests in support of risk assessments at marine and freshwater sites. The projects involved testing whole sediment samples with *Hyalella azteca, Chironomus tentans, Ampelisca abdita, and Leptocheirus plumulosus, Lumbriculus variegatus,*and *Eisenia foetida* (soils). Sample preparation, initiation, and completion of sediment toxicity tests. *Leptocheirus plumulosis neonate* enumeration for many samples.

2008-2009: McAllister Point Landfill, Test America / US Navy
Supported sediment assessments with amphipods *Ampelisca abdita* and *Leptocheirus plumulosus*.

Stuart Randall, Aquatec Biological Sciences, Inc.
273 Commerce Street, Williston, VT 05495
Phone: (802) 860-1638

Resume update: March 2012

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APPENDIX B:
LABORATORY WASTE STORAGE AND DISPOSAL

APPENDIX B:
Laboratory Waste Storage and Disposal

1.0 Introduction

Aquatec Biological Sciences handles and disposes of hazardous waste following U.S. EPA Regulations in 40 CFR 262 and 268 and Vermont Department of Environmental Conservation Regulations in Chapter 7. Laboratory hazardous waste is generated at the facility listed below:

Facility: 273 Commerce Street
Williston, VT 05495
(802) 655-1203

U.S. EPA Generator ID:
VTP000009663

2.0 Category

Presently the laboratory facility generates five hazardous waste categories. The following Table 2.1 lists the categories:

Table 2.1 Waste Categories

Williston (VTP000009663)
Acid
Aqueous (non-regulated)
Mixed Solvent
Soil (non-regulated)
Vials

Employees shall label each 55-gallon drum before transferring material into it. Hazardous waste labels are provided by the hazardous waste treatment, storage and disposal facility (TSDF). Material class labels, such as "Flammable", shall be purchased from a separate vendor. The shipping name, hazard class, waste code and additional information pertinent to each of the above hazardous waste categories are listed on the shipping labels.

3.0 Personal Protective Equipment

Employees removing hazardous waste from the laboratory sections shall wear safety glasses, a laboratory coat and gloves. Additional protective equipment, such as a face shield and apron, shall be worn while transferring acidic and caustic solutions into

drums. Respirators are available for use at the discretion of employees who have been approved.

4.0 Accumulation

Laboratory sections that generate and accumulate hazardous waste and non-regulated waste are listed in Table 4.1.

Table 4.1 Laboratory Sections and Corresponding Waste

Laboratory Section	Category	Description
Toxicology	Aqueous	non-regulated water samples
	Acetone	hazardous waste
	Soil	non-regulated soil samples
	Sediments	non-regulated sediment samples
Ecology	Aqueous	non-regulated water samples
	Soil	non-regulated soil samples
	Sediments	non-regulated sediment samples
	Formalin	regulated waste
Sample Management	Aqueous	non-regulated water samples
	Soil	non-regulated soil samples
	Sediments	non-regulated sediment samples
Microbiology	Aqueous	non-regulated water samples
Limnology	Acetone	hazardous waste

Biologists and technicians shall accumulate hazardous waste and non-regulated waste in containers located at their workstation or stored in sealed containers in laboratory refrigerators. Table 4.2 lists the appropriate container for each hazardous or non-regulated waste category.

Table 4.2 Hazardous waste categories with appropriate container.

Laboratory Section	Category	Original Container
Toxicology	Aqueous	one to five gallon plastic Container/ original container
	Soil and sediment	0.5 gallon to five gallon plastic container/original container
Ecology	Aqueous	Original container
	Soil and sediment	Original container
	Formalin	Original container
Sample Management	Aqueous	original sample container
	Soil and sediment	original sample container
Microbiology	Aqueous	original sample container
Limnology	Acetone	original container

Each container used to accumulate waste at the workstations shall be labeled with at least the name of the waste category.

5.0 Short Term Storage

The transfer of hazardous waste and non-regulated waste from the laboratory will be coordinated with each Department Manager. Non-regulated solid waste is transferred to 55-gallon steel drums stored in the garage. Transport original sample containers of non-regulated waste on a lab cart when a sample holding time has expired, toxicity assessment is completed, or upon clearance by the Department Manager. Transport all other full containers of hazardous waste or non-regulated waste from the work stations to the waste area in a secure transport device such as a rubber bottle carrier or corrosion resistant utility cart designed to contain spills or leaks.

Transfer hazardous waste and non-regulated waste into the appropriate 30 or 55-gallon steel drum. After transferring waste into a drum, secure the bung or cover in place to contain vapors. After each DOT 17E and DOT 6D drum is full, tighten the bung securely with the bung wrench. After each DOT 17H drum is full, place a gasket in the rim of the cover, place the cover over the top of the drum then secure the cover on top of the drum with a drum ring and bolt. Place a sign on the drum indicating that it is full.

6.0 Disposal

As needed, contact the U.S. EPA approved TSDF (currently APT at 893-8281) to schedule shipment of hazardous waste and non-regulated waste for disposal. Report to the TSDF the categories and corresponding number of drums that will be shipped. The TSDF will provide a uniform hazardous waste manifest for waste hauling and disposal documentation. Copies of the uniform hazardous waste manifest are mailed to the state agency responsible for regulating hazardous waste where the TSDF is located, and to the Vermont Department of Environmental Conservation at 103 South Main Street in Waterbury, Vermont. Empty drums are ordered from the TSDF to replace the full drums at the time of waste shipment.

APPENDIX C:
LABORATORY METHODOLOGIES

Aquatec Biological Sciences Laboratory Methodology List

Category: Aquatic Toxicity

Method ID	Title	Reference	SOP Reference
100.1-Pp	Fathead minnow, P. promelas, 10-D survival and growth test for sediments	EPA/600/R-99/064	
* 1000.0	Fathead Minnow, P. promelas, Survival and Growth Test	EPA-821-R-02-013	TOX2-003
1000.0-EC	Fathead Minnow, P. promelas, Survival and Growth Test	EPA-821-R-02-013	TOX2-003
1001.0	Fathead Minnow, P. promelas, Embryo-Larval Survival and Growth	EPA-821-R-02-013	TOX2-014
* 1002.0	Daphnid, C. dubia, Survival and Reproduction Test	EPA-821-R-02-013	TOX2-002
1002.0-EC	Daphnid, C. dubia, Survival and Reproduction Test	EPA-821-R-02-013	TOX2-002
1003.0	Green Alge, Selenastrum capricornutum, GrowthTest	EPA-821-R-02-013	
1004.0	Sheepshead Minnow, C. variegatus, Larval Survival and Growth Test	EPA-821-R-02-014	TOX2-010
1005.0	Sheepshead Minnow, C. variegatus, Embryo-Larval Survival and Growth	EPA-821-R-02-014	
* 1006.0	Inland Silverside, Menidia beryllina, Larval Survival and Growth Test	EPA-821-R-02-014	TOX2-008
* 1007.0	Mysid, Americamysis bahia, Survival, Growth, and Fecundity Test	EPA-821-R-02-014	TOX2-009
1008.0	Sea Urchin, Arbacia punctulata, Fertilization Test Method	EPA-821-R-02-014	TOX2-006
1009.0	Red Macroalga, Champia parvula, Reproduction Test Method	EPA-821-R-02-014	TOX2-007
2000.0-24	Fathead Minnow, P. promelas, 24-H Static Acute Survival	EPA-821-R-02-012	TOX2-011
2000.0-48r	Fathead Minnow, P. promelas, 48-H Renewal Acute Survival	EPA-821-R-02-012	TOX2-011
* 2000.0-48s	Fathead Minnow, P. promelas, 48-H Static Acute Survival	EPA-821-R-02-012	TOX2-011
2000.0-96r	Fathead Minnow, P. promelas, 96-H Renewal Acute Survival	EPA-821-R-02-012	TOX2-011
2000.0-96s	Fathead Minnow, P. promelas, 96-H Static Acute Survival	EPA-821-R-02-012	TOX2-011
2000.0-EC	Fathead Minnow, P. promelas, 48-H EC50		

* NELAC Accredited Methods

Aquatec Biological Sciences Laboratory Methodology List

Category: Aquatic Toxicity

Method ID	Title	Reference	SOP Reference
2002.0-24	Daphnid, C. dubia, 24-H Static Acute Survival	EPA-821-R-02-012	TOX2-001
* 2002.0-48r	Daphnid, C. dubia, 48-H Renewal Acute Survival	EPA-821-R-02-012	TOX2-001
2002.0-48s	Daphnid, C. dubia, 48-H Static Acute Survival	EPA-821-R-02-012	TOX2-001
2002.0-96r	Daphnid, C. dubia, 96-H Renewal Acute Survival	EPA-821-R-02-012	TOX2-001
2002.0-96s	Daphnid, C. dubia, 96-H Static Acute Survival	EPA-821-R-02-012	TOX2-001
2002.0-EC	Daphnid, C. dubia, 48-H EC50		
2004.0-24	Sheepshead Minnow, C. variegatus, 24-H Static Acute	EPA-821-R-02-012	TOX2-016
2004.0-48r	Sheepshead Minnow, C. variegatus, 48-H Renewal Acute	EPA-821-R-02-012	TOX2-016
2004.0-48s	Sheepshead Minnow, C. variegatus, 48-H Static Acute	EPA-821-R-02-012	TOX2-016
2004.0-96r	Sheepshead Minnow, C. variegatus, 96-H Renewal Acute	EPA-821-R-02-012	TOX2-016
2004.0-96s	Sheepshead Minnow, C. variegatus, 96-H Static Acute	EPA-821-R-02-012	TOX2-016
2006.0-24	Inland Silverside, M. beryllina, 24-H Static Acute Survival	EPA-821-R-02-012	TOX2-005
2006.0-48r	Inland Silverside, M. beryllina, 48-H Renewal Acute Survival	EPA-821-R-02-012	TOX2-005
* 2006.0-48s	Inland Silverside, M. beryllina, 48-H Static Acute Survival	EPA-821-R-02-012	TOX2-005
2006.0-96r	Inland Silverside, M. beryllina, 96-H Renewal Acute Survival	EPA-821-R-02-012	TOX2-005
2006.0-96s	Inland Silverside, M. beryllina, 96-H Static Acute Survival	EPA-821-R-02-012	TOX2-005
2007.0-24	Mysid, A. bahia, 24-H Static Acute Survival	EPA-821-R-02-012	TOX2-004
2007.0-48r	Mysid, A. bahia, 48-H Renewal Acute Survival	EPA-821-R-02-012	TOX2-004
* 2007.0-48s	Mysid, A. bahia, 48-H Static Acute Survival	EPA-821-R-02-012	TOX2-004
2007.0-96r	Mysid, A. bahia, 96-H Renewal Acute Survival	EPA-821-R-02-012	TOX2-004

* NELAC Accredited Methods

Aquatec Biological Sciences Laboratory Methodology List

Category: Aquatic Toxicity

Method ID	Title	Reference	SOP Reference
2007.0-96s	Mysid, A. bahia, 96-H Static Acute Survival	EPA-821-R-02-012	TOX2-004
2021.0-Dm24	Daphnid, D. magna, 24-H Static Acute Survival	EPA-821-R-02-012	TOX2-001
2021.0-Dm48r	Daphnid, D. magna, 48-H Renewal Acute Survival	EPA-821-R-02-012	TOX2-001
* 2021.0-Dm48s	Daphnid, D. magna, 48-H Static Acute Survival	EPA-821-R-02-012	TOX2-001
2021.0-Dm96r	Daphnid, D. magna, 96-H Renewal Acute Survival	EPA-821-R-02-012	TOX2-001
2021.0-Dm96s	Daphnid, D. magna, 96-H Static Acute Survival	EPA-821-R-02-012	TOX2-001
2021.0-DmEC	Daphnid, D. magna, EC50		
2021.0-Dp24	Daphnid, D. pulex, 24-H Static Acute Survival	EPA-821-R-02-012	TOX2-001
2021.0-Dp48r	Daphnid, D. pulex, 48-H Renewal Acute Survival	EPA-821-R-02-012	TOX2-001
2021.0-Dp48s	Daphnid, D. pulex, 48-H Static Acute Survival	EPA-821-R-02-012	TOX2-001
2021.0-Dp96r	Daphnid, D. pulex, 96-H Renewal Acute Survival	EPA-821-R-02-012	TOX2-001
2021.0-Dp96s	Daphnid, D. pulex, 96-H Static Acute Survival	EPA-821-R-02-012	TOX2-001
2021.0-DpEC	Daphnid, D. magna, EC50		
Ab 96h-RIM	Mysid Shrimp, A. bahia, 96-h Acute Suspended Particulate Phase Toxicity Test	USACE NED RIM	TOX2-017
ABSQA	Aquatec QA		
ACCWTR	Acclimation Water		
Alk&Hard	Alkalinity and Hardness		TOX1-010,-0
Ap 48h-RIM	Sea Urchin, Arbacia punctulata, 48-h Embryo Development Suspended Particulate Phase Toxicity Test	USACE NED RIM	TOX2-013
Centrifuge RI	RIM Centrifugation	ACE-NERIM	
CHEMSUB	Chemistry Subcontract		
EDTA-Cd	EDTA chelation TIE test, C. dubia	EPA/600/6-91/003	
Elutriate	Elutriate Prep		

* NELAC Accredited Methods

Aquatec Biological Sciences Laboratory Methodology List

Category: Aquatic Toxicity

Method ID	*Title*	*Reference*	*SOP Reference*
Hold	Hold Sample for further Instructions		
ITM98-Pp SPP	Fathead minnow, Pimephales promelas, suspended phase 96-h survival test	ACE/EPA Inland Test	
Lab	Laboratory Water		
Lab salt water	Laboratory water-artificial saltwater		
Mb 96h-RIM	Inland Silverside, M. beryllina, 96-h Suspended Particulate Phase Acute Toxicity Test	USACE NED RIM	TOX2-018
MicroTox	Bioluminescence, V. fisheri, Acute MicroTox - 81.9% Basic Test		TOX2-VF
MLAB	Laboratory Water-Moderately Hard		
OECD-201	Green Algae, P. Subcapitata, Growth Inhibition Test	OECD/OCDE	TOX2-PS
OECD-202	Daphnid, D. magna, 48-H Static Acute Survival and Normality	OECD/OCDE	
OECD-203	Fathead Minnow, P. promelas, 96-H Static Acute Survival	OECD/OCDE	
OECD-221	Duckweed, L. minor, Growth Inhibition Test	OECD/OCDE	TOX2-LM
OECD-222	Earthworm, Eisenia fetida, 56-day Survival and Reproduction Test	OECD/OCDE	TOX3-Eiseni
Receiving	Receiving Water		
Renewal	Renewal water		
Ship Cooler	Ship Cooler		
SLAB	Laboratory Water-Soft		
TOX	Tox Operations		

Aquatec Biological Sciences Laboratory Methodology List

Category: Bioaccumulation

Method ID	*Title*	*Reference*	*SOP Reference*
100.3	Oligochaete, L. variegatus, Bioaccumulation Test for sediments	EPA/600/R-99/064	TOX3-006
Lv-Screen	Oligochaete, L. variegatus, Screening Test	EPA/600/R-99/064	
Mn 28d-RIM	Macoma Clam, M. nasuta, 28-Day Bioaccumulation Test	USACE NED RIM	TOX3-012
Nv 28d-RIM	Polychaete, N. virens, 28-Day Bioaccumulation Test	USACE NED RIM	TOX3-013

Aquatec Biological Sciences Laboratory Methodology List

Category: Ecology

Method ID	*Title*	*Reference*	*SOP Reference*
10200H3-C	Chlorophyll a - Corrected		ECO-005
10200H3-U	Chlorophyll a - Uncorrected		ECO-005
Biomass	Sample Biomass		

Aquatec Biological Sciences Laboratory Methodology List

Category: Fisheries

Method ID	*Title*	*Reference*	*SOP Reference*
Base-LT	Base Monitoring-Lake Trout		
CSMI-FF	CSMI-Forage Fish Monitoring Initiative		
CSMI-LT	CSMI-Lake Trout and Stomach Monitoring Initiative		
EQBLK	Equipment Blank		
FishID	Fish Identification		
Homog	Homogenization		ECO-007
Tissue	Preparation of organism tissue for chemical analysis		ECO-007

Aquatec Biological Sciences Laboratory Methodology List

Category: Microbiology

Method ID	*Title*	*Reference*	*SOP Reference*
2540G	Percent Solids	STD Methods	
9215B	Heterotrophic Plate Count	STD Methods	HPC
9221E-S	Fecal Coliform Sludge MPN, MPN/g (dry)	STD Methods	FC Sludge M

Aquatec Biological Sciences Laboratory Methodology List

Category: Sediment Toxicity

Method ID	*Title*	*Reference*	*SOP Reference*
100.1	Amphipod, H. azteca, 10-D Survival and Growth Test for Sediments	EPA/600/R-99/064	TOX3-001
100.2	Midge, C. tentans, 10-D Survival and Growth	EPA/600/R-99/064	TOX3-004
100.4	Amphipod, H. azteca, 42-D Survival, Growth, and Reproduction Test	EPA/600/R-99/064	TOX3-003
100.4-28Ha	Amphipod, H. azteca, 28-D Survival and Growth Test	EPA/600/R-99/064	TOX3-016
100.4-Aa	Amphipod, A. abdita, 10-D Survival	EPA/600/R-94/025	TOX3-009
100.4-Lp	Amphipod, L. plumulosus, 10-D Survival	EPA/600/R-94/025	TOX3-008
100.5	Midge, C. tentans, Life-cycle Test for Measuring the Effects of Sediment-associated contaminants on Chironomus tentans	EPA/600/R-99/064	TOX3-005
100.5-28Ct	Midge, C. tentans, 28-Day Survival and Emergence Test for Sediments	EPA/600/R-99/064	TOX3-018
100.5-42Ct	Midge, C. tentans, 42-Day Survival, Growth, Emergence, and Reproduction Test	EPA/600/R-99/064	TOX3-020
Aa 10d-RIM	Amphipod, A. abdita, 10-Day Sediment Toxicity Test	USACE NED RIM	TOX3-009
Aa-Screen	Amphipod, A. abdita, Screening Test		
Ab 10d-RIM	Mysid Shrimp, A. bahia, 10-Day Sediment Toxicity Test	USACE NED RIM	TOX3-011
Ammonia	Ammonia		
Composite	Composite		
Ct-Screen	Midge, C. tentans, Screening Test	EPA/600/R-99/064	
Ha-Screen	Amphipod, H. azteca, Screening Test	EPA/600/R-99/064	
Lp-10	Leptocheirus plumulosus 10-day Survival Test	ACE-NERIM	TOX3-008
Lp-28	Amphipod, L. plumulosus, 28-D Survival, Growth and Reproduction	EPA/600/R-01/020	TOX3-017
LP-Screen	Amphipod, L. plumulosus, Screening Test		
POREX	Pore water extraction		

Aquatec Biological Sciences Laboratory Methodology List

Category: Taxonomy

Method ID	*Title*	*Reference*	*SOP Reference*
10200F	Phytoplankton Identification and Enumeration		ECO-002
10200G	Zooplankton Identification and Enumeration		ECO-003
IchthyoID	Ichthyoplankton Identification and Enumeration		ECO-004
MacroID	Macroinvertebrate Identification and Enumeration		ECO-001

Aquatec Biological Sciences Laboratory Methodology List

Category: Terrestrial Toxicity

Method ID	*Title*	*Reference*	*SOP Reference*
Ef-Screen	Earthworm, E. foetida, Screening Test		

APPENDIX D:

Certifications and Accreditations

CERTIFICATIONS

The following is a list of professional certifications and scored Quality Assurance documents currently held for biological analyses.

DMR

Toxicology

Aquatec participates in the annual USEPA DMR Whole Effluent Toxicity (WET) Quality Assurance Program. The program is currently administered by a private firm. Aquatec currently contracts with Environmental Resource Associates (ERA) of Arvada, Colorado for reference standards and reporting.

ACCREDITATION

Aquatec is a NELAP-Accredited laboratory for toxicity testing. Primary accreditation was awarded through the State of New Hampshire Environmental Laboratory Accreditation Program, in accordance with NELAC Standards (Certificate Number: 173704).

OSHA

Select Aquatec employees hold current certification for OSHA 29 CFR 1910.120 HAZWOPER training, mostly required for fieldwork at sites with potential hazardous waste contact.

State of New Hampshire
Environmental Laboratory Accreditation Program

Awards
PRIMARY ACCREDITATION
to

AQUATEC BIOLOGICAL SCIENCES

of

WILLISTON, VT

For the analytes listed on the attached page(s) in accordance with the provisions on the NELAC Standards and Env-C 300.

Certificate Number: 173711
Effective Date: July 6, 2011
Expiration Date: July 5, 2012
Laboratory ID: 1737

NELAP RECOGNIZED

Bill Hall
NH ELAP Program Manager

Method accreditation does not imply acceptance for NHDES compliance testing. Laboratory is required to use EPA-approved methods where required by regulation. Continuing accreditation status is dependent on successful ongoing participation in the program. Customers may verify the lab's current accreditation status by calling (603) 271-2998.

NEW HAMPSHIRE ENVIRONMENTAL LABORATORY ACCREDITATION PROGRAM

29 Hazen Drive, PO Box 95, Concord, NH 03302 (603) 271-2998

PRIMARY ACCREDITATION PARAMETER LIST

ANALYTE LIST NUMBER: 173711-A

NELAP RECOGNIZED

AQUATEC BIOLOGICAL SCIENCES
273 COMMERCE ST
WILLISTON VT 05495
(802) 860-1638
Lab ID 1737

Method Code / Analyte Code	Method Reference / Analyte Name	Revision	Revision Date: Effective Date	Revision Date: Expiration Date	Matrix Code	Category	Accr. Type
10215404	**EPA 2021.0 EPA/821/R-02/012**	**5TH ED**	**OCT-02**				
3350	DAPHNIA MAGNA		Jul 30, 2008	Jul 5, 2012	N	WET	NE
10215608	**EPA 2021.0 EPA/821/R-02/012**	**5TH ED**	**OCT-02**				
3355	DAPHNIA PULEX		Jul 30, 2008	Jul 5, 2012	N	WET	NE
10216407	**EPA 2006.0 EPA/821/R-02/012**	**5TH ED**	**OCT-02**				
3380	MENIDIA BERYLLINA (INLAND SILVERSIDE)		Jul 30, 2008	Jul 5, 2012	N	WET	NE
10252605	**EPA 1000.0 EPA/821/R-02/013**	**4th ED**	**OCT-02**				
3410	PIMEPHALES PROMELAS (FATHEAD MINNOW)		Jul 30, 2008	Jul 5, 2012	N	WET	NE
10252809	**EPA 1001.0 EPA/821/R-02/013**	**4th ED**	**OCT-02**				
3410	PIMEPHALES PROMELAS (FATHEAD MINNOW)		Jul 30, 2008	Jul 5, 2012	N	WET	NE
10253006	**EPA 1002.0 EPA/821/R-02/013**	**4th ED**	**OCT-02**				
3315	CERIODAPHNIA DUBIA (DAPHNID)		Jul 30, 2008	Jul 5, 2012	N	WET	NE
10253802	**EPA 1006.0 EPA/821/R-03/014**	**3rd ED**	**OCT-02**				
3380	MENIDIA BERYLLINA (INLAND SILVERSIDE)		Jul 30, 2008	Jul 5, 2012	N	WET	NE
10254009	**EPA 1007.0 EPA/821/R-03/014**	**3rd ED**	**OCT-02**				
3395	MYSIDOPSIS BAHIA (MYSID)		Jul 30, 2008	Jul 5, 2012	N	WET	NE
10254203	**EPA 1008.0 EPA/821/R-03/014**	**3rd ED**	**OCT-02**				
3305	ARBACIA PUNCTULATA (SEA URCHIN)		Jul 30, 2008	Jul 5, 2012	N	WET	NE
10264809	**EPA 2000.0 EPA/821/R-02/012**	**5TH ED**	**OCT-02**				
3410	PIMEPHALES PROMELAS (FATHEAD MINNOW)		Jul 30, 2008	Jul 5, 2012	N	WET	NE
NH0114	**EPA 2007.0 EPA/821/R-02/012**	**5TH ED**	**OCT-02**				
3395	MYSIDOPSIS BAHIA (MYSID)		Jul 30, 2008	Jul 5, 2012	N	WET	NE
NH0116	**EPA 2002.0 EPA/821/R-02/012**	**5TH ED**	**OCT-02**				
3315	CERIODAPHNIA DUBIA (DAPHNID)		Jul 30, 2008	Jul 5, 2012	N	WET	NE

This analyte list supersedes all previously issued analyte lists. Method accreditation does not imply acceptance for NHDES compliance testing. Customers may verify the laboratory's current accreditation status by calling at (603) 271-2998. Laboratory is required to use EPA approved/accepted methods where required by regulation.

ERA
A Waters Company

John Williams
Aquatec Biological Sciences
273 Commerce St
Williston, VT 05495

DMR-QA 31 Final Report

DMR-QA Proficiency Testing

DMR-QA Study

Open Date: 03/14/11

Close Date: 07/01/11

Report Issued Date: 07/22/11

ERA

A Waters Company

Report Recipient	Contact/Phone Number	Reporting Type
No Recipients Selected		

ERA

A Waters Company

DMRQA-31 Definitions & Study Discussion

Study Dates: 03/14/11 - 07/01/11

Report Issued: 07/22/11

DMRQA Study Definitions

The Reported Value is the value that the laboratory reported to ERA.

The ERA Assigned Values are compliant with the most current USEPA/NELAC FoPT tables. The assigned values are directly traceable to the commercially prepared starting materials used to manufacture the PT standards. A parameter not added to the standard is given an Assigned Value of "0" per the guidelines contained in the USEPA's Criteria Document and NELAC standards.

The Acceptance Limits are established per the criteria contained in the most current USEPA/NELAC FoPT tables, or ERA's SOP for the Generation of Performance Acceptance Limits™ as applicable.

The Performance Evaluation:

Acceptable	= Reported Value falls within the Acceptance Limits.
Not Acceptable	= Reported Value falls outside the Acceptance Limits.
No Evaluation	= Reported Value cannot be evaluated.
Not Reported	= No Value reported.

The Method Description is the method the laboratory reported to ERA.

DMRQA Study Discussion

ERA's DMR-QA 31 Proficiency Testing study has been reviewed by ERA senior management and certified compliant with the requirements of the USEPA's National Standards for Water Proficiency Testing Studies Criteria Document (December 1998), and the criteria contained in the most current NELAC FoPT tables.

ERA's DMR-QA 31 study standards were examined for any anomalies. A full review of all homogeneity, stability and accuracy verification data was completed. All analytical verification data for all analytes met the acceptance criteria contained in the USEPA's National Criteria Document for Water Proficiency Testing Studies, December 1998, and the criteria contained in the most current NELAC FoPT tables.

The data submitted by participating laboratories was also examined for study anomalies. There were no anomalies observed during the statistical review of the data.

ERA's DMR-QA 31 study reports shall not be reproduced except in their entirety and not without the permission of the participating laboratories. The report must not be used by the participating laboratories to claim product endorsement by any agency of the U. S. government.

The data contained herein are confidential and intended for your use only.

If you have any questions or concerns regarding your assessment in ERA's DMRQA Proficiency Testing program, please contact Jay McBurney, Quality Program Manager, or the proficiency testing department at 1-800-372-0122.

ACCREDITED
Lab Code: 1139-01

ERA
A Waters Company

Study: **DMR-QA 31**

ERA Customer Number: **A662354**

Laboratory Name: **Aquatec Biological Sciences**

WET Results

DMR-QA 31 Final Complete Report

ERA
A Waters Company

John Williams
Aquatec Biological Sciences
273 Commerce St
Williston, VT 05495
802-860-1638

EPA ID: VT00908
ERA Customer Number: A662354
Report Issued: 07/22/11
Study Dates: 03/14/11 - 07/01/11

Anal. No.	Test End Point	Reported Value %	Assigned Value %	Acceptance Limits %	Performance Evaluation	Method Description
DMRQA Fathead minnow (Test Code 13) (cat# WET002)						
48Hr., Acute, Non-Renewal, 25° C, MHSF						
Potassium chloride						
0754	LC50	61.6	52.9	28.4 - 77.4	Acceptable	EPA 2000
DMRQA Fathead minnow (Test Code 15) (cat# WET004)						
7-day Short term Chronic, Daily Renewal, MHSF						
Potassium chloride						
0808	IC25 (ON) Growth	29.9	31.7	25.2 - 38.2	Acceptable	EPA 1000
0810	NOEC (ON) Growth	25.0	25.0	12.5 - 50.0	Acceptable	EPA 1000
0756	NOEC Survival	25.0	25.0	12.5 - 50.0	Acceptable	EPA 1000
DMRQA Ceriodaphnia dubia (Test Code 19) (cat# WET008)						
48Hr., Acute Renewal, 25° C, MHSF						
Potassium chloride						
0764	LC50	48.7	40.4	10.0 - 70.8	Acceptable	EPA 2002
DMRQA Ceriodaphnia dubia (Test Code 21) (cat# WET010)						
7-day Short term Chronic, Daily Renewal, MHSF						
Potassium chloride						
0767	IC25 Reproduction	23.8	25.2	12.2 - 38.1	Acceptable	EPA 1002
0768	NOEC Reproduction	12.5	25.0	12.5 - 50.0	Acceptable	EPA 1002
0766	NOEC Survival	25.0	25.0	12.5 - 50.0	Acceptable	EPA 1002
DMRQA Daphnia magna (Test Code 32) (cat# WET012)						
48Hr., Acute, Non-Renewal, 25° C, MHSF						
Potassium chloride						
0788	LC50	55.5	55.1	29.7 - 80.4	Acceptable	EPA 2021
DMRQA Mysid (Test Code 42) (cat# WET016)						
48Hr., Acute, Non-Renewal, 25° C, 40 FSW						
Potassium chloride						
0798	LC50	36.0	36.1	28.2 - 44.0	Acceptable	EPA 2007
DMRQA Mysid (Test Code 43) (cat# WET017)						
7-day Short term Chronic, Daily Renewal, 40 FSW						
Potassium chloride						
0816	IC25 (ON) Growth	31.2	30.8	22.7 - 38.8	Acceptable	EPA 1007
0818	NOEC (ON) Growth	25.0	25.0	12.5 - 50.0	Acceptable	EPA 1007
0799	NOEC Survival	25.0	25.0	12.5 - 50.0	Acceptable	EPA 1007
DMRQA Inland silverside (Test Code 44) (cat# WET018)						
48Hr., Acute, Non-Renewal, 25° C, 40 FSW						
Potassium chloride						
0803	LC50	70.1	70.4	49.9 - 90.9	Acceptable	EPA 2006

ACCREDITED Lab Code: 1539-01

All analytes are included in ERA's A2LA accreditation. Lab Code: 1539-01

DMR-QA 31 Final Complete Report

A Waters Company

John Williams
Aquatec Biological Sciences
273 Commerce St
Williston, VT 05495
802-860-1638

EPA ID: VT00908
ERA Customer Number: A662354
Report Issued: 07/22/11
Study Dates: 03/14/11 - 07/01/11

Anal. No.	Test End Point	Reported Value %	Assigned Value %	Acceptance Limits %	Performance Evaluation	Method Description
DMRQA Inland Silverside (Test Code 45) (cat# WET013)						
7-day Short term Chronic, Daily Renewal, 40 FSW						
Potassium chloride						
0825	IC25 (ON) Growth	60.2	58.3	48.8 - 67.8	Acceptable	EPA 1006.0
0826	NOEC (ON) Growth	25.0	50.0	25.0 - 100	Acceptable	EPA 1006.0
0824	NOEC Survival	50.0	50.0	25.0 - 100	Acceptable	EPA 1006.0

All analytes are included in ERA's A2LA accreditation. Lab Code: 1539-01

APPENDIX E:
Figures

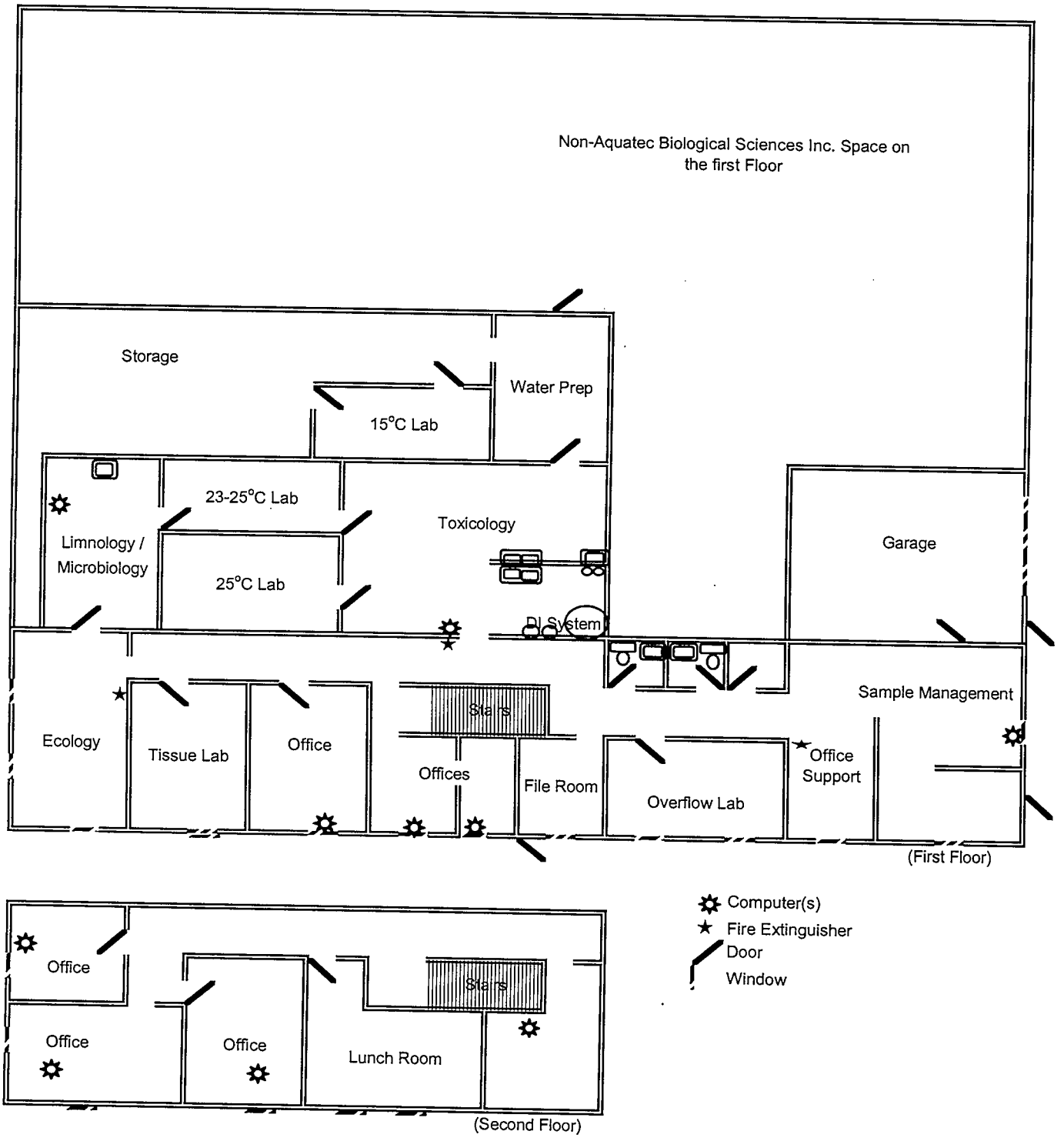

Figure 1. Floor plan for Aquatec Biological Sciences

Demonstration of Capability (Initial Training)
Test Method: 1000.0

Laboratory Name: Aquatec Biological Sciences, Inc.

Analyst's Name (s):

Department: Environmental Toxicology

Matrix: [X] Water

Method: (1000.0, Fathead minnow *Pimephales promelas* 7-d Survival and Growth):

Associated SOP: TOX2-003
Note: this method requires several supporting elements of training assessment and experience. Document completion of training for components of the method (See checklist below). When training has been satisfactorily completed for all components, the DOC for the overall method should be signed below and the analyst may perform the overall method without direct supervision.

The analyst(s) identified above, using the cited test method(s) which is in use at this facility for the analyses of samples under the National Environmental Laboratory Accreditation Program, have met the Demonstration of Capability.

1) The test method(s) was performed by the analyst(s) identified on this form.

2) Raw data (including a copy of this form) necessary to reconstruct and validate these analyses are attached.

Technical Director: John Williams Date:

QA Officer: Philip Downey, Ph.D. or Jennifer Garrison, Deputy QA Officer Date:

Procedure	Trainer/Trainee Initials	Date
Alkalinity and hardness		
Total residual chlorine (flask and DPD)		
Sample prep		
Balance operation		
WQ (DO, pH, conductivity, temperature		
Start test (organism prep and distribution)		
Daily renewal, organism counts, observations		
Feeding		
End test, prep pans, dry wts.		

Figure 2. Example of an Analyst Initial Demonstration of Capability

Equipment Repair and Maintenance Log

Date	Description of Equipment	Nature of Maintenance or Malfunction	Repair Date or Resolution or Correction of Malfunction	Initials

Figure 3. Example of Equipment Maintenance and Repair form

Corrective Action Report

Date: | **Contact:**

Initiated by: | **SDG:**

Summary of Problem:

Summary of Investigation and Findings:

Resolution/Recommended Corrective Action:

Date of Implementation:

Followup:

Date:

Follow-Up Findings:

Lab Manager Review and Approval:

Figure 4. Example of Corrective Action Report

AQUATEC BIOLOGICAL SCIENCES, INC.
CLIENT COMPLAINT INFORMATION

DATE OF COMPLAINT:

PERSON RECEIVING COMPLAINT:

CLIENT / PERSON REGISTERING COMPLAINT

METHOD OF COMMUNICATION:

Phone: ☐

E-mail: ☐

Other:

DISCRIPTION OF COMPLAINT

ACTIONS TO BE TAKEN TO RESOLVE COMPLAINT

FOLLOW-UP INFORMATION / RESOLUTION

SUBMIT TO DEPARTMENT MANAGER AND DIRECTOR

Director / Manager / QA Officer Review and Approval:

SIGNATURE / DATE:

Figure 5. Example of Client Complaint Form

Aquatec Biological Sciences, Inc.
Internal Chain of Custody Form

Organization	: Aquatec Biological Sciences, Inc.	**BTR Number**	:	1442
Date Received	: 1/1/97	**No. of Samples**	:	2
Project Reference	: Anytown Remediation Project	**Project Number**	:	98000
SDG Number	: XX0001	**Reporting Level**	:	4

Sample Number	Refrigerator Asset #	Date/Time Removed	Reason for Removal	Initials	Refrigerator Asset #	Date/Time Returned	Initials

Figure 6. Example of Internal Chain of Custody Form

Aquatec Biological Sciences

Chain-of-Custody Record

273 Commerce Street
Williston, VT 05495
TEL: (802) 860-1638
FAX: (802) 658-3189

COMPANY INFORMATION	COMPANY'S PROJECT INFORMATION	SHIPPING INFORMATION	VOLUME/CONTAINER TYPE/ PRESERVATIVE (NOTE 4)
Name: _____	Project Name: _____	Carrier: _____	
Address: _____			
_____	Project Number: _____	Airbill Number: _____	
_____	Sampler Name(s): _____		
Telephone: _____		Date Shipped: _____	
Facsimile: _____			
Contact Name: _____	Quote #:_____ Client Code: _____	Hand Delivered: Q Yes Q No	

SAMPLE IDENTIFICATION (NOTE1)	COLLECTION DATE	COLLECTION TIME	GRAB	COMPOSITE	MATRIX	ANALYSIS/REMARKS (NOTE 2,3)	NUMBER OF CONTAINERS

Relinquished by: *(signature)*	DATE	TIME	Received by: *(signature)*
Relinquished by: *(signature)*	DATE	TIME	Received by: *(signature)*
Relinquished by: *(signature)*	DATE	TIME	Received by: *(signature)*

NOTES TO SAMPLER(S): (1) Limit Sample Identification to 30 characters, if possible; (2) Indicate designated Lab Q.C. sample and type (e.g.:MS/MSD/REP) and provide sufficient sample; (3) Field duplicates are separate sample; (4) e.g.: 40 ml/glass/H_2SO_4

Notes to Lab:

Distribution: Original Accompanies Shipment; Copy to Coordinator Field Files

Figure 7. Generic Chain-of-Custody Form

Aquatec Biological Sciences, Inc.
Chain-of-Custody Record

273 Commerce Street
Williston, VT 05495
TEL: (802) 860-1638
FAX: (802) 658-3189

COMPANY INFORMATION	COMPANY'S PROJECT INFORMATION	SHIPPING INFORMATION
Name: Client A Address: 222 Pleasant Street ~~Suite 102~~ City/State/Zip: A town in, MA 01xxx Telephone: ( ) Facsimile: Contact Name: Ms. Contact	Project Name: **Outfall Composite** Project Number: 11xxx Sampler Name(s): Client Code: XXXX	Carrier: Airbill Number: Date Shipped: Hand Delivered: ☐ Yes ☐ No

SAMPLE IDENTIFICATION	COLLECTION DATE	COLLECTION TIME	GRAB	COMPOSITE	MATRIX	ANALYSIS	VOLUME/CONTAINER TYPE/ PRESERVATIVE: NUMBER OF CONTAINERS 4°C / Plastic / 1 gal	4°C / Plastic / 1/2	4°C H_2SO_4 / Plastic / 1 L	4°C H_2SO_4 / Glass / 40	4°C / Amber Glass / 250	4°C / Plastic / 0.5 L
Outfall Composite					Effluent	*Daphnia pulex* 48-h Static Acute Toxicity (EPA Method 2021.0)	1					
Outfall Composite					Effluent	Total Residual Chlorine					1	
Pristine River					Receiving	Dilution Water	1					
Pristine River					Receiving	Total Residual Chlorine					1	

Relinquished by: *(signature)*	DATE	TIME	Received by: *(signature)*
Relinquished by: *(signature)*	DATE	TIME	Received by: *(signature)*
Relinquished by: *(signature)*	DATE	TIME	Received by: *(signature)*

Temperature blank at delivery: °C.

Remarks:
Chem Sub: deliver chemistry subsamples to analytical lab

Sample acceptance policy:
Documentation: completed date/time of sample collection and Sampler's name on this COC.
Labels: Completed date/time sample collected/initials on each label. Cover label with waterproof tape.
Bottles: Tape caps of 1-gal bottles (toxicity samples) to prevent dislodgement.
Sample holding time: 36 hours completion of collection to first use. Samples used beyond prescribed holding times will be qualified in the report.
Ship samples on ice (ice should be bagged): Samples should arrive in range of 0 – 6°C. Samples received outside of prescribed temperatures will be qualified in the report.

Figure 8. Example of Project-specific Chain-of-Custody Form

Figure 9
AQUATEC BIOLOGICAL SCIENCES, INC.
ANNUAL MANAGEMENT REVIEW

In accordance with NELAC 2003, Section 5.4.14, Aquatec's Executive Management shall at least annually conduct a review of our quality system and our environmental testing activities. The purpose of this review is to ensure continuing suitability and effectiveness of our laboratory methods and our overall operation. This review will also point to the potential need to introduce new changes or improvements in our operation. The attached checklist has been prepared as a guideline for the annual review however the review may also incorporate elements of our operation beyond the checklist.

Date of Annual Management Review:

Summary of Overall Findings:

Management Review Signature:

Philip C. Downey, Ph.D.
Director

ANNUAL MANAGEMENT REVIEW CHECKLIST

Review Element	Y / N / NA	Findings / Comment / Corrective Action
1. Are Aquatec's policies and procedures up-to-date and suitable?		Findings & Comments; Corrective Action:
2. Are reports from Laboratory Managers or Supervisors reviewed?		Findings & Comments; Corrective Action:
3. Have the outcomes of annual or recent internal audits been reviewed and addressed?		Findings & Comments; Corrective Action:
4. Have any corrective or preventative actions been documented and reviewed?		Findings & Comments; Corrective Action:
5. Have assessments by external auditors, agencies, or clients been reviewed and addressed?		Findings & Comments; Corrective Action:
6. Have the results of interlaboratory comparisons or proficiency tests been reviewed and have any issues or inconsistencies been addressed?		Findings & Comments; Corrective Action:
7. Have any changes in the volume and type of work been evaluated?		Findings & Comments; Corrective Action:
8. Has client feedback, if received, been reviewed? If negative feedback has been received, have issues been resolved?		Findings & Comments; Corrective Action:
9. Have any clients submitted complaints, and if so have the issues been evaluated and resolved?		Findings & Comments; Corrective Action:
10. Have other QA activities, resources, and staff training been reviewed and remained current?		Findings & Comments; Corrective Action:

Additional Annual Management Review Considerations:

Review Element	Y / N / NA	Findings / Comment / Corrective Action
11. Has the QAPP been reviewed and updated?		Findings & Comments; Corrective Action:
12. Have technical reports been reviewed? (e.g., at least once per Quarter)		Findings & Comments; Corrective Action:
13. Has annual integrity training been completed?		Findings & Comments; Corrective Action:
14. Was there any evidence of inappropriate actions or vulnerabilities related to data integrity?		Findings & Comments; Corrective Action:
15. Has a check on the validity of our statistical program (CETIS) been completed this year?		Findings & Comments; Corrective Action:
16.		Findings & Comments; Corrective Action:
17.		Findings & Comments; Corrective Action:
18.		Findings & Comments; Corrective Action:
19.		Findings & Comments; Corrective Action:
20.		Findings & Comments; Corrective Action:

APPENDIX F
MASTER LIST OF EQUIPMENT

(NOTE: ADDITIONAL INFORMATION AND TRACKING OF MAINTENANCE AND REPAIRS IS MAINTAINED IN THE ACCESS DATA BASE FOR EQUIPMENT.)

Aquatec Biological Sciences Equipment List

Category: Ecology

Equipment Discription	Manufacturer	Number of units
15 Cu Ft Freezer	Maytag	1
15 Cu Ft Freezer	Maytag	1
15 Cu Ft Freezer	Maytag	1
15 Cu Ft Freezer	Maytag	1
15 Cu Ft Freezer	Maytag	1
ALPKEM FS3000	O.I. Analytica	1
Brinkman PT3000 Tissue Homogenizer	Brinkman	1
Bueler Bone saw	Bueler	1
Eberbach Fish Scale Projector	Eberbach	1
Fiber-Lite #9745-00 Fiber Optic Illuminat	Cole-Parmer	5
Fish Scale Press	Ann Arbor	1
Hydrolab Scout 2 Multiparameter field W	Hydrolab	1
Labconco Biological Safety Glove Box	Labconco	1
Leica APO Dissecting Microscope	Leica	1
LEM Meat Ginder #32 - 1.5HP	LEM	1
Magnifier light		4
Olympus IM Photography Microscope	Olympus	1
OMNI Tissue Homogenizer	Omni	2
Safeaire 5' Chemical Hood	Fisher Hamilt	1
Spectronic Genesys 5	Spectronic	1
Spencer AO Dissecting Microscope	Spencer	1
Swiftcam 2	Swiftcam	1
TD Model 700 Fluorometer	Turners Desi	1
Tensor Magnifiers	Tensor	2
Zeiss Compound Microscope	Zeiss	1
Zeiss Compound Microscope	Zeiss	1
Zeiss Stemi SR Dissecting Microscope	Zeiss	1
Zeiss Stemi SV6 Dissecting Microscope	Zeiss	1

Aquatec Biological Sciences Equipment List

Category: Field

Equipment Discription	Manufacturer	Number of units
2003 GMC Envoy	GMC	1
A65 Chart Plotter	Raymarine	1
Aluminum boat-12'	Unknown	1
Angler 20' Boat	Angler	1
Boston Whaler-12'	Boston Whal	1
Clarke-Bumpus Plankton Net		1
Coffelt Electrofisher-18'	Coffelt	1
Coffelt Electrofisher-18'	Coffelt	1
Ekman Dredges (9" Sq.)		2
EPIRG SwitLik Survival Raft	SwitLik	1
Fish Experimental Gill Nets		15
Fish Trap Nets		6
Furano Fathometer	Furano	1
Furano Radar	Furano	1
ISCO Model 3700 Water Sampler	ISCO	1
Jon Boat/Merc Motor-18'	Mercury	2
Magnavox MX200 GPS	Magnavox	1
Metered Ichthyoplankton Nets		3
Peterson Dredge		1
Phyto/Zooplankton nets		6
Ponar Dredge (6" sq.)		1
Ponar Dredge (9" sq.)		1
Portable balance, LS2000	Ohaus	1
Seines (up to 100')		3
Smith - Root LR 24 backpack electro sho	Smith - Root	1
Survey Boat-Surf Hunter-25'	Fairhaven Ma	1
Time Delta-C Ultrasonic Flowmeter	Fuji Electroni	1
Yamaha 150 HP outboard engine	Yamaha	1

Aquatec Biological Sciences Equipment List

Category: Microbiology

Equipment Discription	Manufacturer	Number of units
Barnant Temperature Recorder 1	Barnant	1
Hot Plate Model PC-351	Corning	1
Hot Plate Model PC-351	Corning	3
Market Forge Sterilmatic	Market Forge	1
O'Haus Precision Plus Balance Model TP	O Haus	1
Precision 5300 Water Bath	Precision	6
Precision Scientific Incubator Model 805	Precision	1
VWR 1500E Incubator	VWR	1

Aquatec Biological Sciences Equipment List

Category: Office

Equipment Discription	Manufacturer	Number of units
Dell Demension 4600 PC	Dell	1
Dell Dimension 8200	Dell	1
Dell Dimension 8250	Dell	1
Dell Dimension PST 500	Dell	1
Dell Dimension XPS T500	Dell	1
Dell Power Edge 2500 Server-RAID 5	Dell	1
Dell Vostro Computers	Dell	2
HP Laser Jet M3035	Hewlett-Pack	1
HP LaserJet 1200 Series	Hewlett-Pack	1
HP LaserJet 4 Plus	Hewlett-Pack	1
HP Pavilion g6-1d60us Notebook PC	Hewlett-Pack	1
HP Pavilion zv5255us	Hewlett-Pack	1
HP Presario - Laptop CQ60	Hewlett-Pack	1

Aquatec Biological Sciences Equipment List

Category: Sample Management

Equipment Discription	Manufacturer	Number of units
12 Cu Ft. Freezer	Kenmore	1
GBC - Report binder	General Bindi	1
Hobart 3-door SS Refrigerator	Hobart	2
IBM Wheelwriter3		1
Magic Chef Refrigerator	Magic Chef	1
Salter Brecknell PS150	Salter Breckn	1
Traceable to NIST Digital Thermometer	Traceable	8

Aquatec Biological Sciences Equipment List

Category: Toxicology

Equipment Discription	Manufacturer	Number of units
15 Cu Ft Freezer	GE	1
2 kg Weight	Mettler Toled	1
Barnant Temperature Recorder 2	Barnant	1
Barnant Temperature Recorder 3	Barnant	1
Cold spot Compact Refrigerater	Cold Spot	1
Damon/IEC HNSII Centrifuge	Damon/IEC	1
Desk Top Small Lightbox	Logan	3
Dissolved oxygen probe	YSI	1
Drying Oven VWR 1320	VWR	1
ERTCO -L Certified Thermometer 001	ERTCO	1
ERTCO -L Certified Thermometer 002	ERTCO	1
ERTCO -L Certified Thermometer 003	ERTCO	1
IEC B-22M Centrifuge	IEC	1
Isotemp Magnetic Mixer	Fisher	2
LB 100 Large Lightbox	Apollo	2
Lightnin Mixer EV6P25	Lightnin	1
Lightnin Mixer Model EV1P25	Lightnin	1
Magic Chef Refrigerator	Magic Chef	1
Metler PM4600 Balance	Mettler	1
Mettler AE100 Balance	Mettler	1
Mettler M3 Balance	Mettler	1
Microbics M500 Toxicity Analyzer	Microbics	1
Model 145 Conductivity Meter	Thermo Orio	1
Model 40 pH Meter	Beckman	1
Model 7 pH Meter	Corning	1
Model 7 pH Meter	Corning	1
NanoPure water purifier	Barnstead	1
Orbital shaker	Eberbach	1
Orbital shaker	Lab Line	1
Pipetor: 1000uL (fixed)	Oxford	2
Pipetor: 100-1000uL	Finnpipette	2
Pipetor: 10-100uL	Eppendorf	2

Aquatec Biological Sciences Equipment List

Pipetor: 10mL (fixed)	Finnpipette	1
Pipetor: 150uL (fixed)		1
Pipetor: 1-5mL	Oxford	2
Pipetor: 2-20mL (fixed)	Finnpipette	1
Pipetor: 50-1000uL	Eppendorf	2
Pipetor: 5-10mL	Oxford	3
Pocket Colorimeter II	HACH	1
Precision Low Temperature Incubator	Precision	2
RO Water System	Culligan	1
Sorval RC-3B Dynac Refrigerated Centrif	Sorval	1
Thermix 120 MR Magnetic Mixer	Fisher	1
Thermix 120 S Magnetic Mixer	Fisher	1
Thermolyne 1300 Furnace FB1315M	Thermolyne	1
Traceable to NIST Digital Thermometer	Traceable	1
Traceable to NIST Digital Thermometer	Traceable	1
Traceable to NIST Digital Thermometer	Traceable	1
Traceable to NIST Digital Thermometer	Traceable	1
Troemner/26 Reference Weights	Troemner	1
YSI Model 58 DO Meter	YSI	1

Standard Operating Procedure for Amphipod, *Hyalella azteca,* 10-day Survival and Growth Toxicity Test for Sediments EPA Method 100.1

1.0 OBJECTIVE

This SOP describes procedures for performing a ten-day whole sediment survival and growth toxicity test. This test is used to estimate the toxicity of whole sediment samples to the freshwater amphipod, *Hyalella azteca*. When required, toxicity is estimated by statistical comparisons to the control sediment and/or reference sediment. This procedure is based on the guidelines of EPA/600/R-99/064: *Methods for Assessing the Toxicity of and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates*, Method 100.1.

WARNING: Samples acquired for toxicity testing may contain unknown toxicants or health hazards. Lab coats and protective eyeware and gloves should be worn when handling samples.

2.0 PREPARATION

Equipment and Apparatus

Calibrated Instrumentation and Water Quality Apparatus:
- pH meter
- Dissolved Oxygen (DO) meter
- Conductivity meter
- Thermometer (accurate to 0.1°C)
- Alkalinity and hardness titration apparatus
- Mettler M3 Microbalance
- VWR 1320 drying oven
- Ammonia analyzer (Alpchem Enviroflow FS3000)

Additional Equipment:
- Test chambers (300-ml beakers with overflow screen, 8 per sample)
- Automated water-delivery system
- Disposable polyethylene or glass pipets
- Light tables
- Waste collection bucket
- Carolina bowls (assorted sizes)
- Nitex mesh sieves
- Dessicator
- Colored tape
- Aluminum weigh pans
- Forceps

Reagents:
- Reconstituted moderately hardwater (EPA/600/R-99/064)
- Deionized water
- 70 percent Ethanol
- Reference toxicant solutions (KCl)

Forms and Paperwork:
- Amphipod (*Hyalella azteca*) Data Package

2.1 Test System and Conditions

The test system and environmental conditions for the 10-day survival and growth test are summarized in Table 1.

2.2 Test Organisms

Procurement and Documentation

Amphipods are obtained from a commercial supplier or from in-house cultures. If possible, schedule delivery of amphipods approximately 24 hours prior to test initiation. Order sufficient organisms for 10 amphipods per replicate (80 per test sample) and a surplus for reference toxicant testing and intial dry weight determination. Request that the supplier provide information regarding the age and environmental conditions for the test organisms.

Amphipods are shipped by next-day carrier and delivered to Aquatec Biological Sciences. The amphipods are typically shipped in a 500-mL plastic container. Upon receipt , examine the organisms and document their apparent condition, as well as the dissolved oxygen (D.O.), pH, temperature and conductivity of the shipping water. Record the observations on the Organism Data Sheet provided by the supplier. Place a copy of this sheet in the project data package. File the original in the current year organism tracking file.

Evaluation of Amphipod Condition

If, during examination, it appears that more than 5% of the organisms have died during transport, or if the temperature or other environmental conditions are widely different from test requirements, notify the Toxicity Laboratory Manager immediately. A decision will be made regarding the possibility of obtaining a new stock of organisms for testing. If the test is to be delayed, document the reason on the Project Documentation form. Also, it may be necessary to notify the client.

Acclimation and Holding

Transfer the amphipods to a plastic or glass container. Add incremental amounts of laboratory reconstituted water and acclimate to test temperature (23°C). Provide aeration to the holding container. Overlying water temperature should not be changed more than 3°C per day. Monitor organism mortality, temperature, pH, and D.O. during the holding period. Record monitoring data on the Organism Holding and Acclimation form. If more than five percent of the organisms die, contact the Laboratory Manager and arrange for a replacement order.

Food

When holding amphipods prior to testing, feed daily with sufficient *Selenastrum* and YCT to maintain a monolayer of food on the bottom of the container.

Exposure Water

Reconstituted moderately hardwater prepared following the procedure outlined in Section 7.1.3 of EPA/600/R-99/064 will be used as exposure water (overlying water) during the test. Age the exposure water with vigorous aeration for at least one day prior to use in toxicity testing.

3.0 PROCEDURES

3.1 Control Sediment Preparation

Control sediment is natural sediment collected from the Lamoille River in Fairfax, Vermont. The sediment is sieved through a 0.5-mm or a 0.25-mm sieve to remove indigenous organisms prior to use in the test.

3.2 Test Sediment Preparation

1. Remove sediment samples from the sample storage refrigerators.
2. Transfer the sample to the ventilation hood (if required) in the Sample Preparation Laboratory;
3. Homogenize the sediment with a clean plastic spatula or SS mechanical mixer;
4. Examine for presence of indigenous organisms;
5. If no indigenous organisms are apparent (check very carefully for amphipods or predatory organisms), transfer approximately 100 mL aliquots to each of the replicate test chambers;

6. If indigenous organisms (especially predacious insects or amphipods) are present or suspected to be present, remove them with forceps or press sieve sediment through a 1.0 mm Nitex mesh sieve, re-homogenize, then distribute 100-mL aliquots to each of the test replicates. Notify the Laboratory Manager before making a decision to sieve sediments.
7. Record the visual characteristics of each sediment sample on the Sediment Characterization Data form;
8. Remove a subsample of sediment for pore water extraction by centrifugation. Measure the pH of the pore water and preserve it (H_2SO_4) for subsequent total ammonia analysis.
9. Add overlying water to each replicate to a final volume of approximately 275 mL.
10. Return the unused sediment sample to the refrigerator;
11. Randomize the the test chambers positions within the automated water delivery system and begin the water renewal cycles (noon and midnight). The test replicates remain in the test system overnight prior to addition of test organisms.

3.3 Measure Initial Overlying Water Chemistry

On the day of test initiation remove an aliquot of overlying water from representative replicates of each test sample. Measure and record the following parameters: pH, DO, temperature, conductivity, alkalinity, and hardness. Aliquots of overlying water are also stored and preserved for Day 0 ammonia analyses. The temperature of the exposure water should be within the range of 23 ± 1°C. Dissolved oxygen should be above 2.5 mg/L. Additional water exchanges or aeration may be required if dissolved oxygen levels do not remain above 2.5 mg/L.

3.4 Test Initiation: Prepare and Distribute Test Organisms

1. Place the amphipod holding container over a light table and use a large bore disposable polyethylene or glass transfer pipet to transfer amphipods to 1-oz. (30 mL) disposable cups until each cup contains 10 amphipods. Prepare sufficient cups for one for each replicate.
2. A separate analyst should provide a QC check on the count and condition of amphipods in each cup. If descrepancies are noted (counts other than 10, unhealthy appearing), amphipods may be removed or added to that cup to reach 10 active amphipods.
3. Prepare color-coded tags for each replicate.
4. Two analysts are required to start a test. Analyst 1 randomly selects a cup containing amphipods and gently transfers the amphipods into a test replicate using a gentle rinse with a pipet and reconstituted water. **WARNING: Do not dip condiment cups into the exposure water.**
5. Analyst 2 tags the replicate with colored tape to indicate that amphipods have been added to that replicate. Analyst 2 also inspects the holding cup to see whether all amphipods have been transferred to the replicate. Additional rinses are provided by Analyst 1 if needed.
6. This procedure is repeated until all replicates have received organisms (all holding cups should now be empty).
7. Check for amphipods floating on the water surface in each replicate. A drop of exposure water can be used to submerge any amphipods that become trapped on the surface.
8. Record the date and time of test initiation when amphipods have been distributed to all test chambers.
9. Within approximately one hour, re-check all test replicates and submerge any amphipods which are floating or appear dead. Any amphipods observed to be possibly dead or weak-appearing amphipods should be replaced at that time.
10. Set the water delivery apparatus in place and complete the Systems check list.
11. Transfer 10 amphipods (pre-rinsed with deionized water) with forceps to each of eight pre-weighed pans and dry overnight in the VWR oven at approximately 60°C - 90°C. Allow to cool in the dessicator and then weigh them to determine initial dry weight.

3.5 Daily Monitoring

Water Quality Monitoring

The environmental conditions monitoring schedule is outlined in Table 1. Whenever subsamples of overlying water are removed, be sure that no amphipods have been removed from the replicate (Transfer back to replicate if pulled with water sample.). On Days 0 and 9 (or 10) preserve a portion of the overlying

water sample with H_2S0_4 for ammonia-N analysis. These samples should be properly labeled and stored in a refrigerator at 4°C for subsequent analysis.

Biological Monitoring
Examine test beakers daily and record any observations of unusual organism behavior (e.g., sediment avoidance, floating on water surface), if apparent.

Feeding
Provide 1.0 mL of YCT to each test replicate daily.

Automated Water Delivery System
Complete the System Checklist during a delivery cycle daily. The system is designed to provide two overlying water replacements daily, at approximately 12-hour intervals (near noon and near midnight). During the noon renewal, watch the renewal and ensure that all components of the delivery system are functioning properly. Replace any delivery ports that show slow or no delivery due to clogging.

3.6 Termination of the Whole Sediment Toxicity Test

Final Chemistry
Remove an aliquot of exposure water from several test replicates and pool to obtain sufficient water for the Day 10 water chemistry analyses. Check the water to be sure that no amphipods have been removed. Measure and record the final chemistry parameters as specified in Table 1.

Day 10 Survival

1. Transfer a test replicate to a light table equipped with side lighting. Search for amphipods and remove any alive or dead amphipods with a transfer pipet. Decant the overlying water into a Carolina bowl and search for amphipods. After searching the surface layer of sediment, transfer the remaining sediment to a 0.5 mm sieve. Rinse the sediment through the sieve. Pool all amphipods found from a single replicate into a lableled 30-mL disposable cup. Count and record the total number of amphipods surviving on the Survival Data Form. If organisms appear to be dead, examine them under a dissecting microscope. If any movement is detected, the amphipod is considered to be alive.
2. If fewer than 8 amphipods are recovered, a QC check must be performed by another analyst. Any additional amphipods found on the QC must be included in the tally of number surviving.

Day 10 Growth by Dry Weight
Growth is based upon the mean dry weight of surviving amphipods, by replicate. Transfer surviving, deionized water rinsed amphipods to pre-weighed weighing boats and dry overnight in the drying oven at 60° - 90°C. Weigh the boats and the dried amphipods to the nearest 0.01 mg. The Mettler M3 microbalance is used for all dry weight determinations.

Optional Day 10 Growth by Length
Growth may also be assessed using amphipod total body length as the measurement endpoint. Amphipods from a single replicate are transferred to a glass slide and placed on the calibrated projection system. The projected image of each amphipod is measured on a computer screen by tracing along the arc (dorsal surface) of the projected image from head to tail for each amphipod. Final actual amphipod lengths are calculated based on the calibration factor for the projector using an Excel spreadsheet.

4.0 QUALITY ASSURANCE

4.1 Test Acceptability

Test acceptability criteria are based upon the guidelines of EPA/600/R-99/064, Table 11.1. Specifically, a test is judged to be acceptable if the average survival of control amphipods is equal to or greater than 80% at the end of the test. The environmental conditions must be within the tolerance limits of *Hyalella azteca*.

4.2 Protocol Deviations

Any deviations from this SOP should be noted on a project documentation form and the Laboratory Manager and/or the Project Director should be immediately notified. The Project Director will determine the appropriate corrective action and will communicate protocol deviations to the client.

4.3 Reference Toxicant Testing

A water-only 96-hour exposure of amphipods to potassium chloride (KCl) will be conducted concurrently with the sediment exposures and with the same batch of amphipods. The 96-hour LC50 from this standard reference toxicant test is used to assess the sensitivity of the test organisms and to develop a control chart of LC50 values for this species when exposed to potassium chloride.

5.0 SAFETY AND SAMPLE DISPOSAL

Samples acquired for toxicity testing may contain unknown toxicants or health hazards. Lab coats and protective gloves and eyeware should be worn when handling these samples.

Residual sediments remaining after test initiation and completion should held refrigerated for a period of time. The Lab Manager must approve disposal of sediments. Typically, residual sediments are held refrigerated at least until the completion of testing and reports have been compiled. Typically residual sediments are stored refrigerated for several weeks following completion of the tests. The Lab Manager should contact the client to gain approval for sediment disposal. Residual unused sediment is transferred to labeled sediment waste drums for disposal by an authorized waste handler.

6.0 TRAINING

Qualification for the overall procedure outlined in this SOP includes:

Read this SOP.
Receive verbal and visual instruction.
Demonstrate 90% recovery of 10 amphipods from sediment.
Be trained on pertinent associated SOPs.

Table 1. Test conditions for the amphipod *(Hyalella azteca)* 10-day whole sediment survival and growth toxicity test.

ASSOCIATED PROTOCOLS: EPA 2000. *Methods for Assessing the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates* (EPA/600/R-99/064) Method 100.1.

1. Test type:	Whole-sediment toxicity with renewal of overlying water
2. Temperature:	Average 23 ± 1 °C; instantaneous 23 ± 3 °C
3. Light quality:	Wide-spectrum fluorescent lights
4. Illumination:	About 100 to 1000 lux
5. Photoperiod:	16 h light; 8 h dark
6. Test chamber size:	300 ml beaker
7. Sediment volume:	100 ml
8. Overlying water volume:	175 ml
9. Renewal of overlying water:	Two volume additions per day at approximately 12-h intervals (noon and midnight)
10. Life stage of organisms:	Juvenile, 7-14 days old (1-2-day age range) at the start of the test
11. Organisms / test chamber:	10
12. Replicates / sample:	8
13. Feeding:	1.0 ml YCT daily per replicate test chamber
14. Aeration:	None, unless D.O. drops below 2.5 mg/L. Additional overlying water renewals may be preferable to aeration.
15. Overlying water:	Reconstituted moderately hard water
16. Control sediment:	Natural sediment, 0.5 mm sieved
17. Sediment preparation:	Homogenize sediments before distribution to test chambers. Remove any indigenous organisms noted. If required, press sieve (1 mm sieve).
18. Water quality monitoring:	Daily overlying water: temperature, dissolved oxygen Pre-test pore water pH and total ammonia Days 0 and 9 (or 10) overlying water: pH, conductivity, alkalinity, hardness, and ammonia
19. Biological monitoring:	Organism behavior daily
20. Test duration:	10 days
21. Endpoints:	Survival and growth (organism average dry weight), length (optional)
22. Reference toxicant:	Potassium chloride, 96-h acute, water only
23. Test acceptability (control performance):	Minimum mean survival of 80% and measurable growth for the control organisms
24. Data interpretation:	Hypothesis tests versus the control or the reference site responses

DOCUMENT SIGNATURE PAGE

DOCUMENT NAME: SOP TOX3-001 Amphipod, *Hyalella azteca,* 10-day Survival and Growth Rev. 4

Printed Name	I have read and I understand and I agree, to the best of my ability, to follow the procedures outlined in this SOP Signature	Initials	Date
John Williams		JW	5/16/12
Kaitlyn Koch			5/16/12
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Standard Operating Procedure for Midge, *Chironomus dilutus* (formerly *tentans*), 10-day Survival and Growth Toxicity Test for Sediments

1.0 OBJECTIVE

This SOP describes procedures for performing a ten-day whole sediment survival and growth toxicity test. This test is used to estimate the toxicity of whole sediment samples to the freshwater midge, *Chironomus dilutus* (formerly *tentans*). When required, toxicity is estimated by statistical comparisons to the control sediment or reference sediment. This procedure is based on the guidelines of EPA/600/R-99/064: *Methods for Assessing the Toxicity of and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates* Method 100.2.

WARNING: Samples acquired for toxicity testing may contain unknown toxicants or health hazards. Lab coats, protective eyeware and gloves should be worn when handling samples.

2.0 PREPARATION

Equipment and Apparatus

Calibrated Instrumentation and Water Quality Apparatus:

- pH meter
- Dissolved Oxygen (DO) meter
- Thermometer (accurate to 0.1°C)
- Conductivity meter
- Alkalinity and hardness titration apparatus
- Ammonia analyzer (Alpchem Enviroflow FS3000)
- Mettler M3 Microbalance
- VWR 1320 drying oven
- Furnace, Thermolyne 1300

Additional Equipment:

- Test chambers (300-ml beakers with aeration port and overflow screen), 8 per sample
- Aeration system
- Automated water-delivery system
- Large bore glass pipets
- Light tables
- Overlying water collection system
- Carolina bowls
- Nitex mesh sieves (0.5 mm)
- Ceramic crucibles
- Colored tape

Reagents:

- Reconstituted moderately hardwater (EPA/600/R-94/024)
- Deionized water
- 70 percent Ethanol
- Reference toxicant stock solution (KCl)

Forms and Paperwork:

- Midge (*Chironomus dilutus*) 10-day data package

2.1 Test System and Conditions

The test system and environmental conditions for the 10-day survival and growth test are summarized in Table 1.

2.2 Test Organisms

Procurement and Documentation

Midges are obtained from a commercial supplier or from in-house cultures. Approximately 12 days before testing, adult male and female midges are isolated in mating flasks overnight. The next morning, freshly deposited egg cases are transferred to a petri dish containing culture water. After two days (at 23°C) larvae should begin to hatch from the egg case. Egg cases with hatching larvae are transferred to a culture container containing culture water and a monolayer of culture substrate (paper towels or fine and medium sand). Maintain the culture approximately 8-9 days (post-hatch) until the larvae reach second-to-third instar (about 10 days). Organism are acclimated to the exposure water used in testing during the period prior to test initiation.

If midge larvae are acquired from a commercial supplier, they should be scheduled for receipt at least one day prior to initiating tests, if possible. Second-to-third instar (about 9-10 days post hatch) should be used to start the tests, therefore the age should be specified accordingly.

Evaluation of Midge Condition

Examine the condition of the organisms to be used in testing, if it appears that more than 5% of the organisms have died or if the temperature or other environmental conditions are widely different from test requirements, notify the Toxicity Laboratory Manager immediately. A decision will be made regarding the possibility of obtaining a new stock of organisms for testing. If the test is to be delayed, document the reason on the Project Documentation form. Also, it may be necessary to notify the client.

Acclimation and Holding

Midge larvae are held in a plastic or glass container. Provide aeration to the holding container. Overlying water temperature should not be changed more than 3°C per day. Monitor organism mortality, temperature, pH, dissolved oxygen, and conductivity during the growout/acclimation period. Record monitoring data on the *Chironomus dilutus* organism holding form.

Feeding during pre-test holding

Feed Tetrafin suspension daily at a rate so as not to result in accumulation of uneaten food.

Exposure Water

Reconstituted moderately hardwater prepared following the procedure outlined in Section 7.1.3 of EPA/600/R-99/064 will be used as exposure water (overlying water) during the test. Age the exposure water with vigorous aeration for at least one day prior to use in toxicity testing.

3.0 PROCEDURES

3.1 Control Sediment Preparation

Control sediment is natural sediment collected from the Lamoille River in Fairfax, Vermont. The sediment is sieved through a 0.5-mm or a 0.25-mm sieve to remove indigenous organisms prior to use in the test.

3.2 Test Sediment Preparation

1. Remove sediment samples from sample storage refrigerators.
2. If necessary due to possible odors, transfer the sample to the ventilation hood;
3. Inspect the sample for indigenous organisms;
4. If no indigenous organisms are apparent (check very carefully for midges), homogenize and transfer approximately 100 mL aliquots to each of the replicate test chambers;
5. If indigenous organisms (especially predacious insects or midges) are orbserved or suspected to be present, remove them with forceps or press sieve sediment through a 1.0 mm mesh sieve;
6. Homogenize the sediment with a clean plastic spatula or SS mechanical mixer and distribute a 100-mL aliquot to each of the test replicates;
7. Record the visual characteristics of each sediment sample on the Sediment Characterization Data form;
8. Remove a subsample of sediment for pore water extraction (by centrifugation). Measure the pH of the pore water and preserve the remainder with H_2SO_4 for subsequent pore water total ammonia analysis;
9. Add overlying water with a splitter system to a final volume of approximately 275 mL;

10. Transfer the test chambers to the automated water delivery system, set up the aeration system (without starting aeration) and begin the water renewal cycles. The test replicates remain in the test system overnight prior to addition of test organisms;
11. Return the unused sediment sample to sample refrigerator for storage.

3.3 Measure Initial Overlying Water Chemistry

On the day of test initiation, remove an aliquot of overlying water from replicates of each test sample. Measure the following parameters: pH, dissolved oxygen (D.O.), temperature, conductivity, and alkalinity and hardness. Record the data directly on the Monitoring Data Form for Day 0. Aliquots of overlying water are also preserved and stored for Day 0 ammonia analyses. The temperature of the exposure water must be within the range of 23 ± 1°C. Dissolved oxygen should be above 2.5 mg/L. Additional water exchanges may be required if D.O. levels do not remain above 2.5 mg/L.

3.4 Test Initiation: Prepare and Distribute Test Organisms

1. Place the midge holding container over a light table and use a large bore glass pipet to transfer 10 midge larvae directly to each test replicate. Use a counting key to keep track of the number of larvae added to each replicate.
2. Reserve a subsample of larvae for a standard reference toxicant test.
3. Check to be sure that all midges sink to the sediment in the test replicate and begin to burrow. A drop of exposure water can be used to submerge any midges that become trapped on the water surface.
4. Record the date and time of test initiation when midges have been distributed to all test chambers. The test replicates are positioned randomly within the testing system.
5. Within one hour, check all test replicates and replace any midges which are floating or have not borrowed or appear dead.
6. Preserve a representative sample of 10-20 midges with 70% ethanol for determination of instar stage by head capsule measurement.

3.5 Daily Monitoring

Water Quality

The environmental conditions monitoring schedule and list of parameters is outlined in Table 1. Remove a sub-sample of overlying water from a representative replicate for each sample for water quality monitoring. On Days 0 and 9 (or 10), full water quality chemistry is done including alkalinity, hardness, and ammonia-N analysis (preserved with H_2S0_4). The ammonia samples must be properly labeled and may be stored refrigerated for subsequent analysis (within 28 days). Activate the aeration system if declining dissolved oxygen concentrations are observed at any time during the 10-d test. The dissolved oxygen concentration should remain above 2.5 mg/L.

Biological Monitoring

Examine test beakers daily and record any observations of unusual organism behavior.

Feeding

Provide 1.5 mL of Tetrafin slurry (4.0 mg/mL) to each test replicate daily

Automated Water Delivery System

Complete the System Checklist during the noon delivery cycle daily. Ensure that all components of the delivery system are functioning properly. Experience has shown that dissolved oxygen concentrations tend to decline in *Chironomus dilutus* tests.

3.6 Termination of the Whole Sediment Toxicity Test

Final Chemistry (Day 9 or 10)

Decant an aliquot of exposure water from several test replicates and pool to obtain sufficient water for the final water chemistry analyses (collected on Day 9 or 10). Measure and record the final chemistry parameters as specified in Table 1.

Day 10 Survival

1. Decant the overlying water and sediment into a 0.5 mm sieve. Rinse the sediment through the sieve. Pool all recovered midges from a single replicate into a labeled 30-mL disposable cup. Count and record the total number of midges surviving on the Survival and Growth Data Form. If organisms appear to be immobile and discolored, they are considered to be dead and are not included in the survival or growth analysis. If any movement is detected, the midge is considered to be alive.

2. If fewer than 8 midge larvae are recovered, a QC check must be performed by another analyst. Any additional larvae found on the repick must be included in the tally of number surviving.
3. Any observed pupae or emerged adults are recorded as survivors but are not included in the growth analysis.

Day 10 Growth (Ash free dry weight)
Growth is based upon the mean ash-free dry weight (AFDW) of pooled surviving midges, by replicate. Surviving midges in each replicate will be used to determine ash-free dry weights. Rinse the larvae from a replicate to clean off attached debris – use a 0.5-mm sieve to rinse the larvae. Combine the larvae from each replicate in ashed and pre-weighed cruicibles (Each crucible is numbered. This number is used to identify the larvae by test replicate and must be recorded.) and dry the larvae at 60°C for approximately 24 hours (or to constant weight). Weigh each replicate crucible to 0.01 mg on the M3 balance, set to the 2000 mg range. Ash the replicate crucibles and larvae at 550°C in the furnace for 2 hours. Re-weigh the ashed larvae. The tissue mass is the difference between the weight of the dried larvae (plus crucible) and the weight of the ashed larvae (plus crucible). The Mettler M3 microbalance is used for all dry or ash-free weight determinations.

4.0 QUALITY ASSURANCE

Test Acceptability
Test acceptability criteria are based upon the guidelines of EPA/600/R-99/064, Table 12.1. Specifically, a test is judged to be acceptable if the average survival of control midges is equal to or greater than 70% and the average ash-free dry weight of the control organisms is $\geq$0.48 mg/organism at the end of the test. The environmental conditions must be within the tolerance limits of *Chironomus dilutus*.

Protocol Deviations
Any deviations from this SOP should be noted on a project documentation form and the Laboratory Manager and/or the Project Director should be immediately notified. The Project Director will determine the appropriate corrective action and will communicate protocol deviations as qualifiers within the narrative of the report.

Reference Toxicant Testing
A water-only 96-hour exposure of midges to potassium chloride (KCl) is conducted concurrently with the sediment exposures with the same batch of larvae. The 96-hour LC50 from this standard reference toxicant test is used to assess the sensitivity of the test organisms and to develop a control chart of LC50 values for this species when exposed to potassium chloride.

5.0 SAFETY AND SAMPLE DISPOSAL
Samples acquired for toxicity testing may contain unknown toxicants or health hazards. Lab coats, protective eyeware and gloves should be worn when handling samples. Some samples may require handling within a ventilation hood.

Residual sediments remaining after test initiation and completion should held refrigerated for a period of time. The Lab Manager must approve disposal of sediments. Typically, residual sediments are held refrigerated at least until the completion of testing and reports have been compiled. Typically residual sediments are stored refrigerated for several weeks following completion of the tests. The Lab Manager should contact the client to gain approval for sediment disposal. Residual unused sediment is transferred to labeled sediment waste drums for disposal by an authorized waste handler.

6.0 TRAINING
To be qualified for the overall procedure outlined in this SOP, the analyst must:

Read this SOP.
Receive verbal and visual instruction.
Demonstrate 90% recovery of 10 midges from sediment.
Be trained on pertinent associated SOPs.

Figure 1. Test conditions for the midge *(Chironomus dilutus)* 10-day whole sediment toxicity test
ASSOCIATED PROTOCOLS: EPA 2000. *Methods for Assessing the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates* (EPA/600/R-99/064) Method 100.2

1. Test type:	Whole-sediment toxicity with renewal of overlying water
2. Temperature:	Average 23 ± 1 °C; instantaneous 23 ± 3 °C
3. Light quality:	Wide-spectrum fluorescent lights
4. Illumination:	About 100 to 1000 lux
5. Photoperiod:	16 h light; 8 h dark
6. Test chamber size:	300 ml beaker
7. Sediment volume:	100 ml
8. Overlying water volume:	175 ml
9. Renewal of overlying water:	2 volume additions per day
10. Life stage of organisms:	Second- to third-instar larvae
11. Organisms / test chamber:	10
12. Replicates / sample:	8
13. Feeding:	Tetrafin slurry (4 mg/ml), 1.5 ml daily
14. Aeration:	Aerate all replicates if a declining dissolved oxygen trend is observed
15. Overlying water:	Reconstituted moderately hard water
16. Control sediment:	Natural sediment, 0.5 mm sieved
17. Sediment preparation:	Remove any indigenous organisms noted. If required, press sieve (1 mm sieve).Homogenize sediments before distribution to test chambers.
18. Water quality monitoring:	Daily: temperature and dissolved oxygen Days 0 and 9 (or 10): pH, conductivity, alkalinity, hardness, and ammonia
19. Biological monitoring:	Daily: organism behavior
20. Test duration:	10 days
21. Endpoints:	Survival and growth (ash-free dry weight)
22. Reference toxicant:	Potassium chloride, 96-h acute, water only
23. Test acceptability (control performance):	Average survival should be ≥70% and average ash-free dry weight ≥0.48 mg/larvae.
24. Data interpretation:	Hypothesis tests versus the control or the reference site responses

DOCUMENT SIGNATURE PAGE

DOCUMENT NAME: SOP TOX3-004 Midge, *Chironomus dilutus,* 10-day Survival and Growth Rev. 6

Printed Name	I have read and I understand and I agree, to the best of my ability, to follow the procedures outlined in this SOP Signature	Initials	Date
Kaithyn Koch			5/16/12
Jennifer Garrison		JG	5-17-12
John Williams		JW	5/18/12

Standard Operating Procedure for Oligochaete, *Lumbriculus variegatus*, 28-day Bioaccumulation Test for Sediments (EPA Method 100.3)

1.0 OBJECTIVE

This SOP describes procedures for performing a 28-day sediment bioaccumulation test. This test is used to estimate the potential for the test organism to bioaccumulate detectable levels of sediment-borne contaminants. The test species is the freshwater oligochaete, *Lumbriculus variegatus*. An optional preliminary 96-h screening level toxicity test may be performed to identify any sediment samples which cause acute organism mortality and therefore may not support survival of organisms in the bioccumulation test (Samples with significant mortality in the screening test are typically excluded from the bioaccumulation analysis.). Organisms surviving the 28-day exposure are separated from the test sediments and then stored frozen for subsequent tissue preparation (homogenization) and shipment to an analytical chemistry laboratory for tissue residue analysis. This procedure is based on the guidelines of EPA/600/R-99/064 (USEPA, March 2000, 2nd Ed.): *Methods for Assessing the Toxicity of and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates*, Method 100.3.

Chemical analysis of organism tissues is beyond the scope of this SOP.

WARNING: Samples acquired for toxicity testing may contain unknown toxicants or health hazards. Lab coats and protective gloves should be worn when handling samples.

2.0 PREPARATION

Equipment and Apparatus

Calibrated Instrumentation and Water Quality Apparatus:

- pH meter
- Dissolved Oxygen (DO) meter
- Conductivity Meter
- Thermometer (accurate to 0.1°C)
- Alkalinity and hardness titration apparatus
- Ammonia analysis instrumentation
- Mettler PM4600 balance

Additional Equipment:

Test chambers: 10-L glass aquaria with overflow port for bioaccumulation test; 300-mL beakers for screening test

- Water-delivery system
- Plastic scoops / spatulas, SS mixer blades
- SS sieves (1.0 mm, 0.5 mm, and 0.25 mm)
- Water baths, heaters, circulators
- Disposable polyethylene transfer pipets
- Light tables and lamps
- Waste collection bucket
- Carolina bowls (assorted sizes)
- 40-mL vials for tissues (may be supplied by analytical laboratory)

Reagents:

- Reconstituted moderately hardwater (EPA/600/R-99/064)
- Control sediment (e.g., natural sediment, 0.25 - 0.5-mm sieved)
- Deionized water
- Reference toxicant solution (KCl)

Forms and Paperwork:

- Oligochaete (*Lumbriculus variegatus*) 4-day Screening Toxicity Test Data (optional)
- Oligochaete (*Lumbriculus variegatus*) 28-day Biological and Chemistry bench sheet
- Sediment Characterization bench sheet
- Organism Holding and Acclimation bench sheet
- Daily Checklist forms

3.0 TEST SYSTEM AND CONDITIONS

The test system and environmental conditions for the screening toxicity test are summarized in Table 1. The test system and environmental conditions for the 28-day bioaccumulation test are summarized in Table 2.

4.0 TEST ORGANISM ACQUISITION AND HOLDING

Procurement and Documentation

Oligochaetes are acquired from a commercial supplier. They are acclimated to the exposure water used in testing during the period prior to test initiation. Sources of oligochaetes include:
Aquatic Research Organisms, Hampton, NH
Aquatic BioSystems, Fort Collins, CO

Prior to the testing, order sufficient organisms to provide 1-5 grams (wet weight) of oligochaetes per replicate test chamber. Organism loading is dependent upon sediment total organic carbon (TOC) and biomass requirements for chemical analyses. (Typically, the biomass required at the end of the test is a minimum of 5 or more grams from a composite of tissues). Allowance should be made for water carried with the organisms when they are distributed to test chambers (The organisms are not blotted therefore, for the bioaccumulation test, oligochaetes can be added to each replicate at approximately 1.33 X the target stocking weight). Organisms may also be required for the screening toxicity tests and are required for reference toxicant testing. The supplier should provide information regarding the age and environmental conditions for the test organisms.

Oligochaetes are shipped by next-day carrier and delivered to Aquatec Biological Sciences, Inc.. Upon receipt, examine the organisms and document their apparent condition, as well as the dissolved oxygen (D.O.), pH, temperature and conductivity of the shipping water. Record the observations on the Organism Data Sheet provided by the supplier. Place a copy of this sheet in the project data package.

Evaluation of Oligochaete Condition

If, during examination, it appears that more than 5% of the organisms have died during transport, or if the temperature or other environmental conditions are different from test requirements (e.g., dissolved oxygen <4 mg/L, temperature <15°C), notify the Toxicity Laboratory Manager immediately. A decision will be made regarding the possibility of increasing the acclimation time or obtaining a new stock of organisms for testing.

Acclimation and Holding

Transfer the oligochaetes to a 4-L plastic storage container. Add incremental amounts of laboratory reconstituted water and acclimate to test temperature (23°C). Provide aeration to the holding container. Overlying water temperature should not change more than 3°C per day. Monitor organism mortality, temperature, pH, D.O., and conductivity during the holding period and record the data on the Organism Holding and Acclimation form. If it is estimated that more than five percent of the organisms die, contact the Toxicity Laboratory Manager and arrange for a replacement order.

Food

As long as the organism holding period is limited to three or less days, feeding is not required.

5.0 CONTROL SEDIMENT AND OVERLYING WATER

Control Sediment Preparation

Control sediment is natural sediment collected from the Lamoille River/Lake Arrowhead Vermont. This sediment has been demonstrated to provide acceptable survival (based on biomass recovery) during 28-day bioaccumulation tests. The sediment is sieved (0.25-0.5 mm mesh SS sieve) during collection in the field.

Exposure Water

Reconstituted moderately hardwater prepared following the procedure outlined in Section 7.1.3 of EPA/600/R-99/064 will be used as exposure water (overlying water) during the test. Age the exposure water with vigorous aeration for at least one day prior to use in toxicity testing.

6.0 TEST PROCEDURES SCREENING TOXICITY TEST

The screening-level toxicity test is a 4-day exposure of test organisms to sediment. The purpose of the screening toxicity test is to possibly eliminate toxic samples (those that result in significant mortality) from the bioaccumulation analysis. Procedures for the screening toxicity test are outlined in Table 1. Procedures for the 28-day bioaccumulation test are outlined in Table 2.

Sediment Preparation for the Screening test

1. Remove sediment samples from Sample Management refrigerators.
2. If indigenous organisms are observed or if there is a high proportion of vegetative material or detritus, remove with forceps or press sieve sediment through a 0.5 or 1.0 mm Nitex mesh sieve;
3. Homogenize the sediment with a clean plastic spatula or SS mechanical mixer blade;
4. Transfer aliquots of the homogenized sediment in approximately 100 mL aliquots to each of the replicate test chambers;
5. Record the visual characteristics of each sediment sample on the Sediment Characterization Data form;
6. Remove a sub-sample of sediment for pore water total ammonia analysis. Transfer the sediment to 50-mL centrifuge tube and centrifuge 15-20 minutes at ½ to ¾ speed (IEC HN-SII centrifuge) to extract pore water. Measure the pore water pH and preserve a sub-sample (H_2SO_4) for subsequent total ammonia analysis. Store the sample refrigerated until the time of analysis;
7. Add overlying water to a final total volume of approximately 300 mL;
8. Return the unused sediment sample to storage refrigerators;
9. Allow the replicates to incubate overnight within the water renewal system.

Screening Test Initiation: Distribution of Test Organisms

1. The loading rate for the screening toxicity test is 10 organisms per replicate.
2. Transfer the organisms using a disposable pipet to each test chamber.
3. A subsample of oligochaetes should be reserved for a concurrent standard reference toxicant test.
4. Measure the weight (wet and dry) of a subset of organisms used to start the test. This information will be used to estimate loading for the 28-day bioaccumulation test.
5. Record the date and time of test initiation when oligochaetes have been distributed to all test chambers. The test replicates are positioned randomly within the testing system.
6. After approximately one hour, observe test replicates. Oligochaetes should begin exhibiting vertical positioning within the sediments. Maintain notes of any unusual behavioral activities (e.g., sediment avoidance).

Screening Test Overlying Water Chemistry and System Monitoring

The schedule and list of parameters for overlying water chemistry are outlined in Table 1. Full chemistry is collected on Days 0 and 3 (or 4). Dissolved oxygen and temperature are measured daily in a sub-sample of overlying water from one replicate for each sample. Complete the System Checklist daily. Ensure that all components of the delivery system (delivery and drainage) are functioning properly. If dissolved oxygen concentration declines, aeration should be provided to each replicate (minimum D.O. 2.5 mg/L).

Screening Toxicity Test Biological Monitoring

Test organism observations are made daily during the renewal system check.

Screening Toxicity Test Feeding

Oligochaetes are not fed during the screening test.

Screening Toxicity Test Completion

On Day 4, organism survival may be assessed by direct observation in the test beakers. An estimate of the number of surviving organisms (initial n=10) may be made by counting oligochaetes with either vertical or horizontal orientation to the sediments and visible movement. Replicates with no survivors are easily identified by observing an absence of live organisms, either vertically or horizontally oriented. Sieving of sediments through a 0.25 or 0.5 mm sieve can be used to verify the number of surviving organisms. There should be no significant reduction in the number of organisms relative to control survival. If a significant reduction in survival is observed, the client should be contacted to determine whether the bioaccumulation test should proceed for samples exhibiting significant reductions in survival.

7.0 TEST PROCEDURES BIOACCUMULATION TEST

Test Sediment Preparation for the Bioaccumulation Test

Procedures for preparing sediment for the bioaccumulation test are similar to the preparation steps for the screening toxicity test (if performed) with the exception that larger sediment volumes and organism biomass are required.

1. Remove sediment samples from Sample Management refrigerators.
2. If indigenous organisms are observed or if there is a high proportion of vegetative material or detritus press sieve sediment through a 0.5 or 1.0 mm SS mesh sieve;
3. Homogenize the sediment with a clean plastic spatula or SS mechanical mixer blade;

4. Transfer aliquots of the homogenized sediment in approximately 1-1.5 L aliquots to each of the replicate test chambers;
5. Remove a sub-sample of sediment for pore water total ammonia analysis. Transfer the sediment to 50-mL centrifuge tube and centrifuge 15-20 minutes at ½ to ¾ speed (IEC HN-SII centrifuge) to extract pore water. Measure the pore water pH and preserve a sub-sample (H_2SO_4) for subsequent total ammonia analysis. Store the pore water sample refrigerated until the time of analysis. (If this step has already been done in association with the screening toxicity test, this step does not need to be repeated);
6. Record the visual characteristics of each sediment sample on the Sediment Characterization Data form (If this step has already been done in association with the screening toxicity test, this step does not need to be repeated);
7. Add overlying water to a final total volume of approximately approximately 6 L (i.e., up to the drainage port) ;
8. Return the unused sediment sample to storage refrigerators;
9. Transfer the test chambers to the water delivery system and begin the water renewal cycles (two renewals daily). The test replicates remain in the test system overnight without addition of test organisms.
10. Some project-specific protocols may require alternative sediment preparation procedures.

Bioaccumulation Test Initiation: Preparation and Distribution of Test Organisms

1. The organism loading rate for the bioaccumulation test is based upon the results of the sediment total organic carbon (TOC) content. The ratio of TOC in sediment to dry weight of organisms at the start of the test should be no less than 50:1. A minimum of 1 g biomass, or up to approximately 5 g biomass added per replicate. A factor of approximately 1.33% X the target stocking rate adjusts for water carryover.
2. An organism scoop is constructed from a disposable polyethylene transfer pipet to transfer oligochaetes to a tared weigh boat on a balance. Transfer the organisms to the replicate test chamber. Each replicate of that sample will receive approximately the same biomass. Record the biomass (g) added to replicates for each sample.
3. A color-coded tag should be used to mark each replicate as organisms are added.
4. A sub-sample of oligochaetes should be reserved for standard reference toxicant testing (if not completed with a screening toxicity test).
5. Archive (frozen) a subsample (5-10 grams) for baseline (Time 0) tissue residue analysis.
6. Record the date and time of test initiation when oligochaetes have been distributed to all test chambers. The test replicates are positioned randomly within the testing system.
7. After approximately one hour, observe test replicates. Oligochaetes should begin exhibiting vertical positioning within the sediments.

Bioaccumulation Test Overlying Water Chemistry and System Monitoring

The schedule and list of parameters for overlying water chemistry are outlined in Table 2. Full chemistry is collected on Days 0 and 27 (or 28). Dissolved oxygen and temperature are measured daily in a sub-sample of overlying water from one replicate for each sample. Complete the System Checklist daily. Ensure that all components of the delivery system (delivery and drainage) are functioning properly. If dissolved oxygen concentration declines, aeration should be provided to each replicate (minimum D.O. 2.5 mg/L).

Bioaccumulation Test Biological Monitoring

Test organism observations are made daily during the renewal system check. Any unusual behavioral activities are recorded.

Bioaccumulation Test Feeding

Oligochaetes are not fed during the bioaccumulation test.

Bioaccumulation Test Termination of Exposure (Day 28)

1. Set a test replicate over nested sieves (e.g., 0.5 mm nested over a 0.25-mm sieve).
2. Rinse the sediment containing oligochaetes into the 0.5-mm sieve with deionized water. Rinse sediments through the 0.5-mm sieve and quickly transfer organisms and material remaining in the 0.5-mm seive to a holding tray to begin the depuration.
3. Material in the lower 0.25 sieve can be rinsed back into the original test container as a back up source of organisms in case some of them moved through the 0.5-mm sieve.
4. During the depuration period, the process of organism cleaning (separation from other residual material) is continued. This process may require repeated transfer of oligochaetes with a transfer pipet to clean water. Several transfers of organisms to clean water will help to separate oligochaetes from attached detritus.

Transfer surviving oligochaetes to a 1-L beaker of reconstituted water to continue the 6-8 hour depuration period (Section 13.3.7 of EPA/600/R-99/064).

5. Clean-up of oligochaetes may continue throughout the depuration period. The oligochaetes tend to congregate in masses. Repeated swirling and decanting of water will progressively separate them from residual non-target material.
6. If tissue residue analyses are being performed on a single composite oligochaete sample (one per sediment sample) organisms from the replicates for a sample may be combined into a single container for depuration. If chemistry analyses will be performed on individual replicates, the depuration groups should be maintained as separate replicates.

Transfer oligochaetes to vials
After completion of the depuration period, transfer organisms to pre-labeled and pre-weighed sample containers. Transfer the oligochaete mass to the container (e.g., 40-mL vials without preservative), drain off residual water, and record the final wet weight of the test organisms plus vial. Transfer oligochaetes in vials to the freezer and record the date and time.

8.0 QUALITY ASSURANCE

Sample Tracking
Each sediment sample, including the Control, will be assigned a unique sample number which will be used throughout the test and associated with the original sample identification code. At the end of the test, tissues will be assigned a new sample number but will retain the original sample identification code with "-T" for tissue.

Test Acceptability
Test acceptability criteria are based upon the guidelines of EPA/600/R-99/064, Tables 13.1 and 13.4. The environmental conditions must be within the tolerance limits of *Lumbriculus variegatus*.

Protocol Deviations
Any deviations from this SOP should be noted on a project documentation form and the Laboratory Manager and/or the Project Director should be immediately notified. The Project Director will determine the appropriate corrective action and will communicate protocol deviations in the final report. Deviations from protocol will be summarized in the Narrative associated with the report.

Reference Toxicant Testing
A water-only 96-hour exposure (one organism per chamber, Table 9.1 of EPA/600/R-99/064) of oligochaetes to potassium chloride (KCl) will be conducted concurrently with the sediment exposures and with the same batch of oligochaetes. The 96-hour LC50 from this standard reference toxicant test is used to assess the sensitivity of the test organisms and to develop a control chart of LC50 values for this species when exposed to potassium chloride.

5.0 SAFETY

Samples acquired for toxicity testing may contain unknown toxicants or health hazards. Lab coats and protective gloves should be worn when handling these samples.

6.0 TRAINING

To be qualified for the overall procedure outlined in this SOP, the analyst must:

Read this SOP.
Receive verbal and visual instruction.
Be trained on pertinent associated SOPs.

Table 1. Test conditions for the oligochaete *(Lumbriculus variegatus)* 4-day screening toxicity test.
ASSOCIATED PROTOCOL: EPA 2000 (2nd Ed.) Methods for Assessing the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates (EPA/600/R-99/064) Method 100.3.

1. Test type:	Whole-sediment toxicity with renewal of overlying water
2. Temperature:	Average 23 ± 1 °C; instantaneous 23 ± 3 °C
3. Light quality:	Wide-spectrum fluorescent lights
4. Illumination:	About 100 to 1000 lux
5. Photoperiod:	16 h light; 8 h dark
6. Test chamber size:	300-mL beaker with overflow port
7. Sediment volume:	Approximately 100 mL
8. Overlying water volume:	Approximately 175 mL
9. Renewal of overlying water:	Two volume additions per day
10. Life stage of organisms:	Adult
11. Organisms / test chamber:	10
12. Replicates / sample:	4
13. Feeding:	None
14. Aeration:	Not required, unless D.O. drops below 2.5 mg/L, however if D.O. shows declining trend, aeration is recommended
15. Overlying water:	Reconstituted moderately hard water
16. Control sediment:	Natural sediment 0.25-0.5-mm sieved
17. Sediment preparation:	Homogenize sediments before distribution to test chambers. Remove any indigenous organisms noted. If required, press sieve (0.5-1 mm)
18. Water quality monitoring:	Days 0 and 3 (or 4): alkalinity, hardness, and ammonia, pH, conductivity Daily: temperature, Dissolved oxygen
19. Biological monitoring:	Daily: Organism behavior; survival on Day 4
20. Test duration:	4 days
21. Endpoints:	Survival
22. Reference toxicant:	Potassium chloride, 96-h acute, water only
23. Test acceptability (control performance):	Performance-based criteria (EPA/600/R-99/064 Table 13.4)
24. Data interpretation or analysis:	Statistical comparison to control response

Table 2. Test conditions for the oligochaete *(Lumbriculus variegatus)* 28-day sediment bioaccumulation test.
ASSOCIATED PROTOCOL: EPA 2000 (2nd Ed.) Methods for Assessing the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates (EPA/600/R-99/064) Method 100.3.

1. Test type:	Whole-sediment toxicity with renewal of overlying water
2. Temperature:	Average 23 ± 1 °C; instantaneous 23 ± 3 °C
3. Light quality:	Wide-spectrum fluorescent lights
4. Illumination:	About 100 to 1000 lux
5. Photoperiod:	16 h light; 8 h dark
6. Test chamber size:	10-L glass aquaria with overflow port
7. Sediment volume:	Approximately 1 - 1.5 L
8. Overlying water volume:	Approximately 6 L
9. Renewal of overlying water:	Two volume additions per day
10. Life stage of organisms:	Adult
11. Organisms / test chamber:	1-5 g (wet weight): adjust for water carried with organisms (1.33 X target stocking weight); ratio of sediment TOC to organism dry weight (approximately 50:1), and; biomass target for tissue chemistry.
12. Replicates / sample:	3-5 (depending on project objectives)
13. Feeding:	None
14. Aeration:	Provide aeration if declining D.O. is observed. D.O should be >2.5 mg/L
15. Overlying water:	Reconstituted moderately hard water
16. Control sediment:	Natural sediment 0.25-0.5-mm sieved
17. Sediment preparation:	Homogenize sediments before distribution to test chambers. Remove any indigenous organisms noted. If required, press sieve (0.5 to 1 mm mesh SS sieve).
18. Water quality monitoring:	Days 0 and 27: alkalinity, hardness, and ammonia Daily: temperature and dissolved oxygen Weekly: pH, Conductivity
19. Biological monitoring:	Daily: Organism behavior; recovery on Day 28
20. Test duration:	Sediment exposure 28 days, depuration 6-24 hours
21. Endpoints:	Bioaccumulation exposure (recovery target to meet project analytical DL objectives for target compounds, e.g., 5-10 g of biomass per sample). Depending on project objectives replicate tissue samples may be discrete or composited (to form a single sample).
22. Reference toxicant:	Potassium chloride, 96-h acute, water only
23. Test acceptability:	Performance-based criteria (EPA/600/R-99/064 Table 13.4)

Method 100.3:
Oligochaete, Lumbriculus variegatus, 28-day bioaccumulation test for sediments

Printed Name	I have read and I understand and I agree, to the best of my ability, to follow the procedures outlined in this SOP Signature	Initials	Date
JOHN WILLIAMS		JW	5/23/12
Jennifer Garrison	Jennifer Garrison	JG	5-23-12

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STANDARD OPERATING PROCEDURE

for

METALS DIGESTION

SOP No.: MET-3050
Revision: 12

Effective date: February 25, 2012

Approved by: _____ Supervisor — 1/26/12 Date

_____ QA Manager — 1/26/12 Date

_____ Laboratory Director — 1/26/12 Date

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METALS DIGESTION

1. SCOPE AND APPLICATION

This procedure uses techniques described in method 3050B for acid digestion of sediments, sludges, and soil samples designated for "Total Metals" analysis. One technique is designed for the preparation of samples for analysis by flame AA (Methods 7420-Pb, 7742-Se, and 7062-As) or ICP-OES (methods 6010 and 200.7). Another technique is given for the preparation of samples for analysis by GFAA (see SOP MET-GFAA for methods) or ICP-MS (methods 6020 and 200.8). This procedure is not a *total digestion* technique, but extracts "environmentally available" elements from the sample of interest.

2. METHOD SUMMARY

One-gram equivalent dry weight sediment, sludge, or soil samples are digested with repeated additions of nitric acid (HNO_3) and hydrogen peroxide (H_2O_2). For GFAA and ICP-MS analysis the resultant digestate is reduced in volume while heating and then diluted to a final volume of 100 mL. For ICP-OES and flame AA analysis, hydrochloric acid (HCl) is added to the initial digestate and the sample is refluxed prior to dilution to a final volume of 100 mL.

3. DEFINITIONS

3.1. **Batch** - A batch of samples is a group of environmental samples that are prepared and/or analyzed together as a unit with the same process and personnel using the same lot(s) of reagents. It is the basic unit for analytical quality control.

3.2. **Preparation Batch** - A preparation batch is composed of one to twenty field samples, all of the same matrix, and with a maximum time between the start of processing of the first and last samples in the batch to be 24 hours.

3.3. **Sample**

3.3.1. Field Sample - An environmental sample collected and delivered to the laboratory for analysis; a.k.a., client's sample.

3.3.2. Laboratory Sample - A representative portion, aliquot, or subsample of a field sample upon which laboratory analyses are made and results generated.

3.4. **Quality System Matrix** - The *matrix* of an environmental sample is distinguished by its physical and/or chemical state and by the program for which the results are intended. The following sections describe the matrix distinctions. These matrices shall be used for purpose of batch and quality control requirements.

3.4.1. Solids - Any solid sample such as soil, sediment, sludge, and other materials with >15% settleable solids.

3.5. **Laboratory Control Sample (LCS)** - A laboratory blank that has been fortified with target analyte and used to determine that the analysis is in control.

3.6. **Matrix Spike (MS)** - In the matrix spike analysis, predetermined quantities of target analytes are added to a sample matrix prior to sample preparation and analysis. The percent recovery is calculated. The MS is used to evaluate the effects of the sample matrix on the method used for the analysis. The concentration of the spike should be at three to five times the sample result or at levels specified by a project analysis plan.

3.7. **Duplicate Sample (DUP)** - A laboratory duplicate. The duplicate sample is a separate field sample aliquot that is processed in an identical manner as the sample proper. The relative percent difference between the samples is calculated and used to assess analytical precision.

3.8. **Method Blank (MB)** - The method blank is an artificial sample composed of analyte-free water or solid matrix and is designed to monitor the introduction of artifacts into the analytical process. The method blank is carried through the entire analytical procedure.

4. INTERFERENCES

Refer to the determinative method for a discussion of interferences.

5. SAFETY

5.1. All appropriate safety precautions for handling solvents, reagents and samples must be taken when performing this procedure. This includes the use of personnel protective equipment, such as, safety glasses, lab coat and the correct gloves.

5.2. Chemicals, reagents and standards must be handled as described in the CAS safety policies, approved methods and in MSDSs where available. Refer to the CAS Environmental, Health and Safety Manual and the appropriate MSDS prior to beginning this method.

5.3. Hydrochloric and/or Nitric Acid are used in this method. These acids are extremely corrosive and care must be taken while handling them. A face shield should be used while pouring acids. And safety glasses should be worn while working with the solutions. Lab coat and gloves should always be worn while working with these solutions.

6. SAMPLE COLLECTION, PRESERVATION AND STORAGE

6.1. Samples may be collected in plastic or glass jars. Non-aqueous samples are refrigerated at 4 ± 2°C from receipt until analysis.

6.2. The recommended holding time is 6 months from the day of sampling.

7. APPARATUS AND EQUIPMENT

7.1. 125 mL plastic cup beaker cup, calibrated at 50mL and 100mL

7.2. Borosilicate watch glasses

7.3. Block Digester, calibrated to maintain 95°C ± 2°C

7.4. Hot Plates: "Thermolyne Cimerac 3", calibrated to maintain 95°C ± 2°C

7.5. Laboratory balance, top-loader capable of reading 0.01g

7.6. Evergreen disposable tubes 50 ml: an Accuracy and Precision verification check must be made with each new vendor lot prior to use. Refer to the SOP for *Checking Volumetric Labware ADM-VOLWARE*, for further detailed instructions. Performance data must meet the accuracy and precision requirements specified in Table 1 (*ADM-VOLWARE*) for non volumetric Labware used for measuring initial and/or final digestate volumes.

7.7. USS # 10 sieve.

8. STANDARDS AND REAGENTS

8.1. Reagent grade chemicals shall be used in all tests. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lowering the accuracy of the determination. The preparation for all laboratory prepared reagents and solutions must be documented in a laboratory logbook. Refer to the SOP *Reagent/Standards Login and Tracking (ADM-RTL)* for the complete procedure and documentation requirements.

8.2. Reagent water: ASTM Type I water (resistivity $\geq$18 MΩ-cm, conductivity $\leq$0.056 uS/cm).

8.3. Concentrated Nitric Acid: J.T. Baker "Instra-analyzed", Trace Metals Grade

8.4. Concentrated Hydrochloric Acid: EMD GR ACS

8.5. Hydrogen Peroxide (30%): EMD GR ACS

8.6. Metals spiking solutions: Three spiking solutions are needed to prepare the matrix spike sample; SS1, SS2, SS3, and SS6. Follow the formulations laid out on the "Metals Spike Form" (see Attachments for example). These solutions are prepared in acid rinsed Class A volumetric flasks using purchased custom mixed standards or 1000 ppm single analyte

standards. Aliquots are made using acid rinsed Class A volumetric pipettes of the appropriate size.

8.6.1. SS1 (Al, Ag, Ba, Be, Cd, Co, Cr, Cu, Fe, Pb, Mn, Ni, Sb, V, and Zn): Fill a 1000 mL volumetric flask approximately half full with reagent water, add 50 mL of nitric acid and mix. Next add 100 mL of the custom mixed standard (CAS-CAL-14) purchased from "Inorganic Ventures". In addition add 50 mL of 1000 ppm Antimony. Dilute to volume with reagent water, mix thoroughly and transfer to a 1000 mL Teflon bottle for storage. The solution expiration date is determined by the earliest expiration date of any single component in the solution.

8.6.2. SS2 (GFAA As, Cd, Cu, Pb, Se, Tl): Fill a 500 mL volumetric flask approximately half full with reagent water, add 25 mL of nitric acid and mix. Next add 2.0 mL each of 1000 ppm Arsenic, Cadmium, Copper, Lead, Selenium, and Thallium. Dilute to volume with reagent water, mix thoroughly and transfer to a 500 mL Teflon bottle for storage. The solution expiration date is determined by the earliest expiration date of any single component in the solution.

8.6.3. SS3 (As, Se, and Tl): Fill a 500 mL volumetric flask approximately half full with reagent water, add 25 mL of nitric acid and mix. Next add 50 mL each of 1000 ppm Arsenic, Selenium, and Thallium. Dilute to volume with reagent water, mix thoroughly and transfer to a 500 mL Teflon bottle for storage. The solution expiration date is determined by the earliest expiration date of any single component in the solution.

8.6.4. SS4 (B, Mo): Fill a 500 mL volumetric flask approximately half full with reagent water, add 25 mL of nitric acid and mix. Next add 50 mL each of 1000 ppm Boron and Molybdenum. Dilute to volume with reagent water, mix thoroughly and transfer to a 500 mL Teflon bottle for storage. The solution's expiration date is determined by the earliest expiration date of any single component in the solution.

8.7. Metals reference material (ERA Priority PollutnT/CLP Inorganic Soil) for use as the laboratory control sample. The expiration date is assigned by the manufacturer.

8.8. Teflon beads, Teflon boiling chips, or other suitable blank material.

9. PREVENTIVE MAINTENANCE

9.1. All maintenance activities are recorded in a maintenance logbook. Pertinent information must be in the logbook. Maintenance entries should include date, symptom of problem, corrective actions, and description of maintenance, date, and name. The log should contain a reference to return to analytical control.

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9.2. Maintenance for this procedure is generally limited to glassware cleaning, pipet monitoring, and hot plate calibration. Procedures for glassware washing are described in the SOP for Metals Laboratory Glassware Cleaning (MET-GC). Procedures for pipet monitoring are given in the SOP for Checking Volumetric Labware, (ADM-VOLWARE).

9.3. Each hotplate or block digestor is uniquely identified and the temperature is verified with each batch of samples. To perform the verification, a certified thermometer is placed in a container half filled with mineral oil, which is then placed in the center of the hotplate or block digester. A clamp is used to ensure the thermometer does not touch the bottom of the beaker. The temperature is turned to the 95°C setting and the mineral oil is allowed to come to temperature. The analyst will verify that the hotplate gives a temperature of 95°C ± 2°C. If not, the thermostat is adjusted until the thermometer reads and maintains 95°C ± 2°C. The thermostat is then marked to clearly indicate the correct setting to be used during sample digestion. The thermostat position and thermometer reading are recorded for each unit in a logbook.

10. RESPONSIBILITIES

10.1. It is the responsibility of the analyst to perform the analysis according to this SOP and to complete all documentation required for data review. Analysis and interpretation of the results are performed by personnel in the laboratory who have demonstrated the ability to generate acceptable results utilizing this SOP. This demonstration is in accordance with the training program of the laboratory. Final review and sign-off of the data is performed by the department supervisor/manager or designee.

10.2. It is the responsibility of the department supervisor/manager to document analyst training.

11. PROCEDURE

11.1. Record all digestion and sample information on the applicable benchsheet. To assist the analyst, a brief description of the procedure is given on the backside of the benchsheet (see Attachments).

11.2. Mix the sample thoroughly to achieve homogeneity. Sieve if necessary using a USS #10 sieve.

11.3. It can be difficult to obtain a representative sample with wet or damp materials. As per Method 3050B, wet samples may be dried, crushed, and ground to reduce subsample variability, however, drying is not recommended since drying may affect the extraction of the analytes of interest in the sample.

11.4. Weigh approximately 1g of sample into a 125ml plastic beaker cup and record the weight to the nearest 0.01g. For sludge's and sediments that have high moisture content, use more sample. A plastic 10.0 mL disposable pipette is used to measure 10.0 mL of sample. The volume and weight of the pipetted sample is recorded. In cases where the sludge is very

thick a 10.0 mL graduated cylinder may be used. The objective is to use about 1g of dry weight sample. For analysis of Lead by Flame AA, use about 2.5g of dry wt. sample and change the final dilution volume to 50ml. This will achieve a lower detection limit needed for most projects. At this point add the appropriate spiking solutions directly onto the designated spike sample prior to addition of reagents.

11.5. Add 5ml reagent water and 5ml concentrated HNO_3. Cover and reflux (without boiling) at 95ºC for 10 to 15 minutes. Allow the sample to cool. Add 5ml of concentrated HNO_3, cover and reflux for 30 minutes. If brown fumes are generated, indicating oxidation of the sample by HNO_3, repeat the addition of 5ml of HNO_3 and reflux over and over until no brown fumes are given off. Reduce the digestate volume to approximately 5 mL without boiling or digest for two hours maintaining a covering of solution over the bottom of the beaker at all times. If this occurs discard the digestate and begin with a new sample aliquot.

Note: The 95°C hotplate temperature must be monitored and documented on a per-batch basis.

11.6. Cool the sample and add 3 mL of 30% H_2O_2. Cover and heat to start the peroxide reaction. Care must be taken to ensure that losses do not occur due to excessive effervescence. Heat in the hot block until effervescence subsides. Remove from hot block and cool the beaker.

11.7. Continue to add 30% H_2O_2 in 3ml aliquots with warming until the effervescence is minimal, or until the general sample appearance is unchanged. Do not add more than 10ml of 30% H_2O_2. When the peroxide additions are complete cover the sample with a watch glass and continue heating the acid-peroxide digestate until the volume has been reduced to approximately 5 mL or heat at 95°± 5°C without boiling for 2 hours. Do not let the samples go to dryness, by ensuring the solution covers the bottom of the vessel at all times.

11.8. If the sample is being prepared for analysis by ICP-OES or Flame AA, add 10 mL of concentrated HCl. If the sample is being prepared for ICP-MS or GFAA analysis no HCl is added. Dilute the sample to 100 mL with reagent water: ASTM Type I water (resistivity $\geq$18 MΩ-cm, conductivity $\leq$0.056 uS/cm) in a 125 mL plastic beaker cup.

Note: for ICP-MS digestates, the tubes must be pre-rinsed with 25% HCl.

Note: For method 7062 and 7742 samples, the 3050B soil digestion is modified as follows: After the final peroxide addition (i.e. before the final reduction stage) add 5.0mL of concentrated hydrochloric acid and reduce the digestate volume to less than 5.0mL, but not to dryness. After cooling, dilute the digestate to 100mL with reagent water.

11.9. Cover and reflux the Flame AA and ICP samples for 15 minutes at 95^{o}C. After cooling, the samples may be diluted to 100ml for ICP analysis, or 50ml for Flame AA analysis.

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11.10. Particulates in the digestates that may clog the nebulizer are allowed to settle overnight, or the digestates may be centrifuged.

11.11. To improve the solubility for Antimony, Barium, Lead and Silver, the following modification of the digestion procedure may be used as directed by the client or project chemist.

11.11.1. Weigh (to the nearest 0.01g) 1.00 g of sample into a 125ml plastic cup. For sludge's and sediments that have high moisture content, use more sample. The objective is to use about 1g of dry weight sample.

11.11.2. Add 2.5mL HNO_3 and 10mL HCl and cover with a watch glass. Reflux for 15 minutes.

11.11.3. Filter the digestate through Whatman No. 41or equivalent filter paper and collect in a 100mL volumetric flask. Wash the filter paper, while still in the funnel, with no more than 5mL of hot (95°) HCl, and then with 20mL of hot (95°) reagent water. Collect washing in the same volumetric flask.

11.11.4. Remove the filter and residue from the funnel, and place them back in the beaker. Add 5mL HCl, cover and heat at 95° ± 5° until the filter paper dissolves. Remove from the heat and wash the cover and sides with reagent water.

11.11.5. Filter the residue and collect the filtrate in the same 100mL flask. Allow to cool, then dilute to volume.

11.11.6. If precipitation occurs in the flask upon cooling, do not dilute to volume. Instead, add up to 10mL of HCl to dissolve the precipitate. After precipitate is dissolved, dilute to volume with water.

12. QA/QC REQUIREMENTS

12.1. Initial Precision and Recovery Validation

12.1.1. The accuracy and precision of the procedure must be validated before analyses of samples begin, or whenever significant changes to the procedures have been made. To do this, four blank matrix samples are spiked with the LCS spike solution, then prepared and analyzed.

12.2. Monitor hotplates on a monthly basis. Report all deficiencies to the Lab Manager. Corrective action must be taken.

12.3. Digest one laboratory control sample with each batch. Weigh 1.00 g of the current lot of Environmental Resource Associates PriorityPollutnT/CLP Inorganic Soil prepared reference material into a 150 mL beaker and digest as per the procedure.

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12.4. Digest one preparation blank (method blank) per digestion batch, or per 20 samples whichever is more frequent. For the method blank, use Teflon beads, Teflon boiling chips, or other suitable solid blank material and follow the digestion procedures.

12.5. Digest one duplicate and one spiked sample with each sample matrix. Prepare one duplicate and spike sample per each digestion batch, or per twenty samples whichever is more frequent. At times, specific samples will be assigned as duplicates of spikes depending on client requirements.

12.6. Soil spikes are prepared by adding 2.0 mL of spike solution #1, and 1 mL each of spike solutions 5 and 6 directly to the sample aliquot, prior to the addition of any water or acid. Fill out a spiking data sheet and keep it with the digestion data sheets.

For ICP and ICP-MS digestions 2.0 mL of SS1 and 1.0 mL of SS5 and SS6 are added to the sample aliquot designated as the matrix spike sample. For GFAA digestions 2.0 mL of SS4 is added to the sample aliquot designated as the matrix spike sample. The matrix spike sample is then digested as per the procedure.

13. REPORTING

13.1. Digestion data sheets including weights and volumes used and reagents/acids are completed and a prep run number or batch lot number is assigned and attached to the data sheet. The lot numbers for the reagents used are added to the digestion data sheet (see Attachments).

13.2. Spiking sheets are completed including all spike data and volumes of spiking solutions used (See Attachments).

14. CORRECTIVE ACTION

14.1. Refer to the SOP for *Corrective Action* for procedures for corrective action. Personnel at all levels and positions in the laboratory are to be alert to identifying problems and nonconformities when errors, deficiencies, or out-of-control situations are detected.

14.2. Handling out-of-control or unacceptable data

14.2.1. On-the-spot corrective actions that are routinely made by analysts and result in acceptable analyses should be documented as normal operating procedures, and no specific documentation need be made other than notations in laboratory maintenance logbooks, runlogs, for example.

14.2.2. Documentation of a nonconformity must be done using a Nonconformity and Corrective Action Report (NCAR) when: a) corrective action is not taken or not possible b) corrective action fails to correct an out-of-control problem on a

laboratory QC or calibration analysis c) reanalysis corrects the nonconformity but is not a procedurally compliant analysis.

15. METHOD PERFORMANCE

Available method performance data is given in the reference method. In addition, this procedure was validated through single laboratory studies of accuracy and precision as in the determinative procedure. The method detection limit(s) and method reporting limit(s) are established for the determinative procedure.

16. POLLUTION PREVENTION

It is the laboratory's practice to minimize the amount of solvents, acids and reagent used to perform this method wherever feasible. Standards are prepared in volumes consistent with methodology and only the amount needed for routine laboratory use is kept on site. The threat to the environment from solvent and reagents used in this method can be minimized when recycled or disposed of properly.

17. WASTE MANAGEMENT

17.1. The laboratory will comply with all Federal, State and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions as specified in the CAS EH&S Manual.

17.2. This method uses acid. Waste acid is hazardous to the sewer system and to the environment. All acid waste must be neutralized to a pH of 2.5-12 prior to disposal down the drain. The neutralization step is considered hazardous waste treatment and must be documented on the treatment by generator record. See the CAS EH&S Manual for details.

18. TRAINING

18.1. Training outline

18.1.1. Review literature (see references section). Read and understand the SOP. Also review the applicable MSDS for all reagents and standards used. Following these reviews, observe the procedure as performed by an experienced analyst at least three times.

18.1.2. The next training step is to assist in the procedure under the guidance of an experienced analyst. During this period, the analyst is expected to transition from a role of assisting, to performing the procedure with minimal oversight from an experienced analyst.

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18.1.3. Perform initial precision and recovery (IPR) study as described above for water samples. Summaries of the IPR are reviewed and signed by the supervisor. Copies may be forwarded to the employee's training file. For applicable tests, IPR studies should be performed in order to be equivalent to NELAC's Initial Demonstration of Capability.

18.2. Training is documented following the *SOP for Documentation of Training*.

NOTE: When the analyst training is documented by the supervisor on internal training documentation forms, the supervisor is acknowledging that the analyst has read and understands this SOP and that adequate training has been given to the analyst to competently perform the analysis independently.

19. METHOD MODIFICATIONS

19.1. The method uses 2 mL of water and 3 mL of H_20_2 in step 11.6. The lab does not add the 2 mL of water. 3.0 mL aliquots of 30% H_20_2 in lieu of 1.0 mL aliquots are added subsequently.

20. REFERENCES

Test Methods for Evaluating Solid Waste, Physical/Chemical Methods. EPA SW-846, 3rd Edition, Final Update III, Method 3050B, December 1996.

21. CHANGES SINCE THE LAST REVISION

21.1. Sec 3.1 – 3.4 is new
21.2. Sec 7.1 – updated
21.3. Sec 8.1 is new
21.4. Sec 9.3 – replaced the word calibrated with verified
21.5. Sec 11.4 – second and third sample is new
21.6. Sec 11.6 – changed 2 mL of water followed by H2O2 to 3 mL of 30% H2O2
21.7. Sec 11.7 – changed 1 mL to 3 mL. Last sentence re-worded for clarity
21.8. Sec 11.8 edits for clarity
21.9. Sec 12.1 is new
21.10. Sec 19 is new

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Table A

METALS SPIKING SOLUTIONS CONCENTRATIONS FORM

Solution Name	Element	mLs of 1000ppm Solution	Final Volume	Solution Conc. mg/L	Concentration in the digest mg/L
SS1	HNO3	50.0	1000ml	-	
	Al	100*	1000ml	200	2
	Ag	100*	1000ml	5	0.05
	Ba	100*	1000ml	200	2
	Be	100*	1000ml	5	0.05
	Cd	100*	1000ml	5	0.05
	Co	100*	1000ml	50	0.5
	Cr	100*	1000ml	20	0.2
	Cu	100*	1000ml	25	0.25
	Fe	100*	1000ml	100	1
	Pb	100*	1000ml	50	0.5
	Mn	100*	1000ml	50	0.5
	Ni	100*	1000ml	50	0.5
	Sb	50	1000ml	50	0.5
	V	100*	1000ml	50	0.5
	Zn	100*	1000ml	50	0.5
SS2	HNO3	25.0	500ml	-	
GFAA SPIKE	As	2.0	500ml	4	0.04
	Cd	2.0	500ml	4	0.04
	Pb	2.0	500ml	4	0.04
	Se	2.0	500ml	4	0.04
	Tl	2.0	500ml	4	0.04
	Cu	2.0	500ml	4	0.04
SS3	HNO3	25.0	500ml	-	
	As	50.0	500ml	100	1
	Se	50.0	500ml	100	1
	Tl	50.0	500ml	100	1
SS4	HNO3	25	500ml	-	
	B	50	500ml	100	1
	Mo	50	500ml	100	1

* Denotes volume of mixed stock standard.
** Denotes 10,000 ppm individual stock standards.

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STANDARD OPERATING PROCEDURE

for

MERCURY IN SOLID OR SEMISOLID WASTE

SOP No.: MET-7471
Revision: 15

Effective date: September 25, 2011

Approved by:

Supervisor	Date: 9/2/11
QA Manager	Date: 9/2/11
Laboratory Director	Date: 9/2/11

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MERCURY IN SOLID OR SEMISOLID WASTE

1. SCOPE AND APPLICATION

1.1. This Standard Operating Procedure (SOP) describes the procedure used to determine the concentrations of Mercury in soils, sediments, freeze dried tissues, bottom deposits, and sludge-type materials using Method EPA 7471A or 7471B. If this dissolution procedure is not sufficient to dissolve a specific matrix type or sample, then this method is not applicable for that matrix. Method 7471 is a cold-vapor atomic absorption procedure.

1.2. The Method Reporting Limit (MRL) is 0.02 mg/kg. Equivalent nomenclature for MRL includes Estimated Quantitation Limit (EQL). Therefore, MRL=EQL. The reported MRL may be adjusted if required for specific project requirements; however, the capability of achieving other reported MRLs must be demonstrated. A Method Detection Limit (MDL) of 0.002 mg/kg has been achieved using this procedure.

2. METHOD SUMMARY

A representative aliquot of sample is prepared as described in this procedure. The mercury is reduced to its elemental state and aerated from solution and measured with an atomic absorption spectrometer. The mercury vapor passes through a cell positioned in the light path of the AA where absorbance is measured as a function of mercury concentration.

3. DEFINITIONS

3.1. **Analysis Sequence** - Samples are analyzed in a set referred to as an analysis sequence. The sequence begins with instrument calibration followed by sample digestates interspersed with calibration standards.

3.2. **Independent Calibration Verification** (ICV) - ICV solutions are made from a stock solution which is different from the stock used to prepare calibration standards and is used to verify the validity of the standardization.

3.3. **Matrix Spike** (MS) - In the matrix spike analysis, predetermined quantities of standard solutions of certain analytes are added to a sample matrix prior to sample digestion and analysis. The purpose of the matrix spike is to evaluate the effects of the sample matrix on the methods used for the analyses. Percent recoveries are calculated for each of the analytes detected.

3.4. **Matrix Spike Duplicate** (MSD) - In the matrix spike duplicate analysis, predetermined quantities of standard solutions of certain analytes are added to a sample matrix prior to sample digestion and analysis. The purpose of the matrix spike duplicate is to evaluate the effects of the sample matrix on the methods used for the analyses. Percent recoveries are

calculated for each of the analytes detected. The relative percent difference between the matrix spikes is calculated and used to assess analytical precision.

3.5. **Duplicate Sample** (DUP) - A laboratory duplicate. The duplicate sample is a separate field sample aliquot that is processed in an identical manner as the sample proper. The relative percent difference between the samples is calculated and used to assess analytical precision.

3.6. **Method Blank** - The method blank is an artificial sample designed to monitor introduction of artifacts into the process. The method blank is carried through the entire analytical procedure.

3.7. **Continuing Calibration Verification Standard (CCV)** - A standard analyzed at specified intervals and used to verify the ongoing validity of the instrument calibration.

3.8. **Instrument Blank (CCB)** - The instrument blank (also called continuing calibration blank) is a volume of blank reagent of composition identical to the digestates. The purpose of the CCB is to determine the levels of contamination associated with the instrumental analysis

4. INTERFERENCES

Potassium permanganate is added to eliminate possible interference from sulfide. Samples high in chlorides require additional permanganate because, during the oxidation step, chlorides are converted to free chlorine, which absorbs radiation at 253 nm.

5. SAFETY

5.1. All appropriate safety precautions for handling solvents, reagents and samples must be taken when performing this procedure. This includes the use of personnel protective equipment, such as, safety glasses, lab coat and the correct gloves.

5.2. Chemicals, reagents and standards must be handled as described in the CAS safety policies, approved methods and in MSDSs where available. Refer to the CAS Environmental, Health and Safety Manual and the appropriate MSDS prior to beginning this method.

5.3. Hydrochloric and/or Nitric Acid are used in this method. These acids are extremely corrosive and care must be taken while handling them. A face shield should be used while pouring acids. And safety glasses should be worn while working with the solutions. Lab coat and gloves should always be worn while working with these solutions.

6. SAMPLE COLLECTION, CONTAINERS, PRESERVATION, AND STORAGE

6.1. Glass, plastic, and polytetraflouroethylene (PTFE) containers are suitable in most cases.

6.2. Non-aqueous samples are stored at 4 ± 2 ºC from receipt until analysis, unless otherwise dictated by project specifications.

6.3. Samples must be analyzed within 28 days of sampling.

7. APPARATUS AND EQUIPMENT

7.1. CETAC M-6000A Mercury Analyzer. See Attachments for instrument parameters.

7.2. CPI-Modified Block (Mod Block)

7.3. Pipettors, Eppendorf and Finnpipette fixed and adjustable volume

7.4. Polypropylene graduated cylinders, 25 mL

7.5. 125 ml Digestion Vessel tubes.

7.6. Laboratory balance, top-loader capable of readings .001g (3-place). Mettler, Ohaus, or equivalent.

8. STANDARDS AND REAGENTS

8.1. Reagent grade chemicals shall be used in all tests. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lowering the accuracy of the determination. The preparation for all laboratory prepared reagents and solutions must be documented in a laboratory logbook. Refer to the SOP *Reagent/Standards Login and Tracking (ADM-RTL)* for the complete procedure and documentation requirements.

8.2. Mercury stock solution (1,000 mg/L). Commercially prepared certified solution stored at room temperature. The expiration date determined by manufacturer.

8.3. Mercury working standard (100µg/L). Prepared from the intermediate stock solution listed above. Store at room temperature and prepare a new standard daily.

8.4. Laboratory Control Sample – ERA Priority Pollutant/CLP Inorganic Soil reference material. Store at room temperature in the original container and use the vendor expiration date.

8.5. Matrix spike solution (1 mg/L) – Prepare by making a 1:1000 dilution of the mercury stock solution. Store at room temperature and prepare a new standard monthly.

Note: See section 11.2.2 for details on preparation of calibration and ICV standards. See section 12 for QC sample preparation.

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8.6. Reagent water - ASTM Type II water (laboratory deionized water).

8.7. Acids - Purity of acids must be established by the laboratory as being high enough to eliminate the introduction of contamination above the Method Reporting Limit.

8.7.1. Nitric Acid (HNO_3) 69-70% – JT Baker-Baker Instra-Analyzed® or equivalent.

8.7.2. Sulfuric Acid concentrated (H_2SO_4) – EMD-OmniTrace® or equivalent.

8.7.3. Hydrochloric Acid concentrated (HCL) – VWR – BHD-Aristar® or equivalent.

8.8. Potassium permanganate solution, 5% w/v. To prepare, add 50 g of solid reagent to 1000 mL of D.I. water and place on magnetic stir plate for approximately 30 minutes until dissolved.

8.9. Sodium chloride/hydroxylamine hydrochloride solution, 12% w/v each. To prepare, add 120g sodium chloride and 120 g of hydroxylamine hydrochloride to 1000 mL of D.I. water and place on magnetic stir plate for approximately 15 minutes until dissolved.

8.10. Stannous chloride, 10% w/v in HCl (7% v/v). To prepare, add 100g stannous chloride crystals and 70 mL of concentrated hydrochloric acid in 1000 mL of D.I. water. Seal lid on mixing bottle and shake until the stannous chloride is dissolved.

8.11. Aqua Regia – Prepare immediately before use by carefully adding 3 parts of concentrated HCL to one part of HNO_3.

9. PREVENTIVE MAINTENANCE

9.1. All maintenance activities are recorded in a maintenance logbook kept for each instrument. Pertinent information (serial numbers, instrument I.D., etc.) must be in the logbook. This includes the routine maintenance described in section 9. The entry in the log must include: date of event, who performed the work, and a reference to analytical control.

9.2. CAS staff performs all routine maintenance and troubleshooting. Preventative maintenance activities listed below should be performed when needed as determined by instrument performance (i.e. stability, sensitivity, etc.) or by visual inspection. Repairs of an extraordinary nature may or may not require factory service, depending on the nature of the task.

9.3. Keep the instrument free of dust, deposits, and chemical spills.

9.4. Replace the peristaltic and autosampler rinse tubing.

9.5. Remove and clean the Gas-Liquid Separator.

9.6. Remove, dismantle, and clean the optical cells (sample cell and reference cell) including the sapphire windows.

9.7. Replace the Hg lamp bulb when the lamp current reaches 13 mA.

10. RESPONSIBILITIES

10.1. It is the responsibility of the analyst to perform the analysis according to this SOP and to complete all documentation required for data review. Analysis and interpretation of the results are performed by personnel in the laboratory who have demonstrated the ability to generate acceptable results utilizing this SOP. This demonstration is in accordance with the training program of the laboratory. Final review and sign-off of the data is performed by the department supervisor/manager or designee.

10.2. It is the responsibility of the department supervisor/manager to document analyst training. Documenting method proficiency, as described in the SOP for *Documentation of Training*, is also the responsibility of the department supervisor/manager.

11. PROCEDURE

11.1. Sample Preparation

11.1.1. Mix the sample thoroughly to achieve homogeneity. For soil, sediment, solids, weigh approximately 0.5g of well-homogenized sample and place in the bottom of a 125 ml digestion tube and record the weight to the nearest 0.01g. Add 5.0 mL of reagent water and 5.0 mL of aqua regia, then heat in the Mod Block for 2 minutes at 95ºC.

11.1.2. Cool then add 10 mL of reagent water and 15 mL of potassium permanganate solution. If the purple color does not persist for 15 minutes add additional potassium permanganate until it does so. Any additional potassium permanganate solution must also be added to the blanks and standards in equal proportion. **Note:** Spiking solution is added prior to acidification.

11.1.3. Mix thoroughly and place in the heating block for 30 minutes at 95ºC. The temperature of the block is monitored with a thermometer that is calibrated monthly.

11.1.4. Cool and add 6 mL of sodium chloride-hydroxylamine hydrochloride to reduce the excess permanganate. Perform this addition under a hood as Cl_2 could be evolved.

11.1.5. Add 27 mL of reagent water and the sample is ready for analysis. (The vapor generator does the step of adding the stannous chloride solution automatically.)

11.2. Calibration

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11.2.1. To prepare calibration standards a 10 ppm intermediate stock solution is first prepared by aliquoting 1.0 mL of commercially prepared 1000 ppm stock standard into an acid rinsed 100 mL Class A volumetric flask and diluting to volume with 1% HNO3. This solution must be prepared monthly. Next, a 100 ppb working solution is prepared by aliquoting 1.0 mL of the 10 ppm intermediate stock solution into an acid rinsed 100 mL Class A volumetric flask and diluting to volume with 1% HNO3. This solution must be prepared daily.

Note: All standard aliquots are measured using calibrated fixed or adjustable volume autopipetters or calibrated disposable 5.0 or 10.0 mL pipettes.

11.2.2. Transfer 0, 0.1, 0.25, 0.5, 2.5 and 5.0 mL aliquots of the working solution to a series of labeled 125 ml digestion tubes. Add the appropriate amount of reagent water to bring each bottle to a volume of 5mL. Add 5.0 mL of aqua regia and heat in the heating block for 2 minutes at 95ºC. The final concentrations of the prepared standards are 0, 0.2, 0.5, 1.0, 5.0, 10.0 ppb.

11.2.3. The Initial Calibration Verification (ICV) is prepared by first making a 1000 ppb intermediate solution. 0.10 mL of commercially prepared 1000 ppm stock standard, from a different manufacturer and lot than the calibration standard, is aliquoted into an acid rinsed 100 mL Class A volumetric flask and diluting to volume with 1% HNO_3. This solution must be prepared monthly. Prepare the ICV standard by aliquoting 0.25 mL to a labeled 125 ml digestion tube. Add the 4.75 mL of reagent water and 5.0 ml of Aqua Regia.

11.2.4. Cool and then add 10 mL of reagent water and 15 mL of potassium permanganate solution and return the bottles to the water bath for 30 minutes.

11.2.5. Cool and add 6.0 mL of sodium chloride-hydroxylamine hydrochloride solution. Add 27 mL of reagent water and the standards are ready for analysis.

11.2.6. CETAC Calibration and Sample Analysis

11.2.6.1. Turn on the CETAC instrument, including the Hg lamp, and autosampler. After this is done turn open the operating software (Mercury Analyzer 1.5.1.1).

11.2.6.2. The rinse station for the autosampler turns on automatically, but the peristaltic pump must be started manually. Make sure all sample uptake and drain tubes are placed correctly on the pump and are secured with the appropriate tension. Place the reagent uptake tube in the stannous chloride and start the pump.

11.2.6.3. From the software's main screen select the "Worksheet" button and then the "Template" button. Select the "Kelso Mercury Program".

11.2.6.4. Go to the "Labels" tab and enter the QC and field samples to be analyzed in the appropriate order.

11.2.6.5. Transfer the solutions to be analyzed to labeled 12mL polyethylene test tubes and place them in the appropriate spaces on the autosampler trays.

11.2.6.6. Transfer the calibration blank and standards (0.2, 0.5, 1.0, 5.0, and 10 ppb) from their digestion tubes to the standard tubes located behind the autosampler trays. The calibration blank is placed in the left most tube and the other standards are placed in ascending order to the right.

11.2.6.7. Return to the software and go to the "Analysis" tab. At this point the analysis is ready to begin. Click on the start button. In the dialog box that appears be sure the following are checked:

- Calibrate before first sample.
- New output file before first sample.
- Zero before first sample.

Click start and the analysis will begin.

11.2.7. After the calibration standards have run the software will use linear regression to create a calibration curve based on the concentration and measured absorbance of each standard. The form of regression line is $y = mx + b$. If the correlation coefficient of the curve is greater than 0.995 the analysis will continue, if not the analysis will be terminated and corrective action will be needed by the analyst.

11.3. As the analysis sequence proceeds, next analyze the following QC standards.

- ICV (5.0 ppb standard prepared from a second source)
- ICB
- CCV (5.0 ppb calibration standard)
- CCB
- CRA (0.2 ppb calibration standard)

If either the ICV or CCV are different from their true values by more than 10% the software will terminate the analysis. If either the ICB or CCB is greater than the MRL the software will terminate the analysis. Method 7471A does not contain criteria for the CRA, however, the result must be a positive measured concentration. For 7471B analyses the criteria are 50-150% of the true value. Also, specific project requirements may apply.

Note: For projects falling under DoD QSM requirements, the QSM criteria for CCV standards is ±20% and for ICB and CCB standards no analytes detected > LOD. (The ICV limit is as listed above.)

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11.4. Sample Analysis

11.4.1. The samples are analyzed with the CETAC analyzer in the same manner as the calibration standards. The analyzer does the step of adding the stannous chloride solution automatically. Check the baseline between samples to verify that the spectrometer reading has stabilized at the normal baseline level.

11.4.2. The analytical sequence should be set up to include all samples, QC samples, blanks, and calibration verification standards at necessary intervals. Refer to the SOP for Sample Batches.

11.4.3. Sample digestion batches are analyzed with a set of CCV and CCB standards which are run at the beginning and end of the analytical run and at a minimum every 10 samples during the run. The same criteria listed above are applied to the CCVs and CCBs and if one is found to be outside these limits the analysis is terminated.

12. QA/QC REQUIREMENTS

12.1. Initial Precision and Recovery Validation

12.1.1. Acceptable accuracy and precision of the procedure must be demonstrated before analysis of samples begins, or whenever significant changes to the procedures have been made.

12.1.2. Accuracy and precision is demonstrated by preparing and analyzing four LCS aliquots. The average percent recovery of for each analyte must be within LCS limits and the %RSD within precision limits.

12.1.3. Initial demonstration of capability must be performed by each analyst performing sample analysis and documented in the laboratory records.

12.2. Method Detection Limits

12.2.1. A method detection limit (MDL) study must be undertaken before analysis of samples can begin. To establish detection limits that are precise and accurate, the analyst must perform the following procedure. Spike a minimum of seven blank replicates with a MDL spiking solution near the MRL and analyze. Refer to the CAS *SOP Performing Method Detection Limit Studies and Establishing Limits of Detection and Quantitation* (ADM-MDL).

12.2.2. Calculate the average concentration found (x) in the sample concentration, and the standard deviation of the concentrations for each analyte. Calculate the MDL for each analyte using the correct T value for the number of replicates. The MDL study should be done annually. The MDL study and MDL verification check should be analyzed

annually or whenever there are major changes in the instrument or procedure is implemented.

12.3. For method 7471B, an LLQC sample (a CRA that is carried through the digestion) must be analyzed to verify accuracy at the MRL. The recovery must be 50-150%.

12.4. For method 7471B, Instrument Detection Limit (IDL) studies are performed quarterly. These will be calculated and made available to the analysts.

12.5. Ongoing QC Samples required are described in the CAS-Kelso Quality Assurance Manual, in the *SOP for Sample Batches* (ADM-Batch). For this analysis, these include:

12.5.1. Prepare one method blank (MB) per digestion batch, or per 20 samples, whichever is more frequent. The MB is to be prepared as done with samples. The Method Blank should be < MRL. If the Method Blank is >MRL redigest the associated samples if sample levels are <20x the MB level.

Note: For projects falling under DoD QSM requirements, the QSM criteria for method blanks is no analytes detected > ½ MRL.

12.5.2. Prepare one Laboratory Control Sample (LCS) per digestion batch, or per 20 samples. Weigh 0.25g of the current lot of "Environmental Resource Associates PriorityPollutnT/CLP Inorganic Soil" prepared reference material in to a 125 mL Digestion vessel tube and prepare as per the procedure.

The LCS recovery criteria are 71-128%, unless project-specific or in-house limits are established. For method 7471B, the LCS recovery limits are 80-120%. If statistical in-house limits are used, they must fall within the 80-120% range.

Note: For DoD QSM projects, the QSM LCS criterion is 80-120%. If the LCS fails the acceptance criteria, redigest the batch of samples.

12.5.3. Prepare one sample duplicate and one matrix spike sample per each digestion batch, or per twenty samples, whichever is more frequent. For the matrix spike, add 0.25mL of the matrix spike solution to the designated spike sample, resulting in a spike concentration of 0.5 mg/kg. At times, specific samples will be assigned as duplicates or spikes depending on client requirements.

Note: Duplicate samples are routinely analyzed; however some projects may require a MSD. All DoD projects require a MSD. The MSD sample is prepare as described above.

The RPD criterion for duplicates is 20% RPD. If not, flag the data or redigest samples. A matrix spike recovery criterion is 60-135%, unless project-specific limits are required. For method 7471B, the recovery limits are 80-120%. If statistical in-house limits are used, they must fall within the 80-120% range. For

DoD QSM work, MS recoveries are assessed using the QSM LCS control limits. If the MS (and/or MSD where applicable) recovery is outside acceptance limits proceed with the additional interference tests described in section 12.5.4. Based on results of these tests, the physical nature of the sample (e.g. homogeneity), and any specific project requirements, a determination can then be made as to appropriate corrective action (e.g. redigestion, reporting with a qualifier, alternative methodologies, etc.). If the analyte concentration is >4x the spike level the spike control limit is no longer applicable and no action is required.

Note: For DoD QSM projects, the duplicate RPD limit is 20% and MS recoveries are assessed using the QSM LCS control limits 80-120%.

12.5.4. Interference Tests: Prepare one post spike for every batch of samples and if samples are sufficiently high (10x the MRL/LOQ) a serial dilution. The serial dilution must agree within 10% of the original sample result. Post spike recovery acceptance limits for method 7471A and 7471B are 80-120% for project falling under SW846 Update IV. When both the post spike and dilution tests fail all of the samples in the associated preparation batch must be quantified via Method of Standard Additions (MSA).

13. DATA REDUCTION, REVIEW, AND REPORTING

13.1. It is the analyst's responsibility to review analytical data to ensure that all quality control requirements have been met for each analytical run. Results for QC analyses are calculated and recorded as specified in section 12.

13.2. Record all sample weight, volumes and dilutions on an A.A. benchsheet (see Attachments).

13.3. Solution concentrations are calculated by the Mercury Analyzer software based on the linear regression calibration curve created when the calibration standards are analyzed. The absorbance measured for each sample is applied to the linear regression curve and the final solution concentration is determined and displayed as the instrument result.

13.4. Calculate sample results using the data system printouts and digestion information. The digestion and dilution information is entered into the data system. The data system then uses the calculations below to generate a sample result. Solid samples are reported in mg/Kg:

$$mg/Kg(Sample) = C^{*} \times PostDigestionDilutionFactor \times \frac{DigestionVol(ml)}{Samplewt(g)} \times \frac{1mg}{1000ug} \times \frac{1L}{1000ml} \times \frac{1000g}{1Kg}$$

C*= Concentration of analyte as measured at the instrument in ug/L (in digestate).

NOTE: If results are to be reported on a dry weight basis as required by certain projects, the Sample Wt (g) component of the equation should be the dry-weight derived from a determination of %moisture of a separate aliquot of the sample using the SOP for Total Solids.

13.5. Record all concentrations determined at the instrument and calculate the final results in mg/Kg. Record the final results on the A.A. Benchsheet.

13.6. The data packet for the sequence is submitted for review by supervisor or designee. The results are transferred to the appropriate report form located in the CAS network directory R:\ICP\WIP. Once the results are transferred, the report is reviewed.

13.7. A daily run log of all samples analyzed is maintained. All data should be printed and stored after operator has checked for evenness of burns. A copy of this document will go with each package of Tier III or higher data run that day.

13.8. Refer to the SOP for *Laboratory Data Review Process* (ADM-DREV) for general instructions for data review.

14. CORRECTIVE ACTION

14.1. Refer to the SOP for Corrective Action (ADM-CA) for procedures for corrective action. Personnel at all levels and positions in the laboratory are to be alert to identifying problems and nonconformities when errors, deficiencies, or out-of-control situations are detected.

14.2. Handling out-of-control or unacceptable data

14.2.1. On-the-spot corrective actions that are routinely made by analysts and result in acceptable analyses should be documented as normal operating procedures, and no specific documentation need be made other than notations in laboratory maintenance logbooks, runlogs, for example.

14.2.2. Documentation of a nonconformity must be done using a Nonconformity and Corrective Action Report (NCAR) when:

- Corrective action is not taken or not possible
- Corrective action fails to correct an out-of-control problem on a laboratory QC or calibration analysis.
- Reanalysis corrects the nonconformity but is not a procedurally compliant analysis.

15. METHOD PERFORMANCE

15.1. This method was validated through single laboratory studies of accuracy and precision. Refer to the reference method for additional available method performance data.

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15.2. The method detection limit (MDL), limit of detection (LOD), and limit of quantitation (LOQ) are established using the procedure described in the SOP for *Performing Method Detection Limit Studies and Establishing Limits of Detection and Quantification (ADM-MDL).* Method Reporting Limits are established for this method based on MDL studies and as specified in the CAS Quality Assurance Manual.

16. POLLUTION PREVENTION

It is the laboratory's practice to minimize the amount of solvents, acids and reagent used to perform this method wherever feasible. Standards are prepared in volumes consistent with methodology and only the amount needed for routine laboratory use is kept on site. The threat to the environment from solvent and reagents used in this method can be minimized when recycled or disposed of properly.

17. WASTE MANAGEMENT

17.1. The laboratory will comply with all Federal, State and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions as specified in the CAS EH&S Manual.

17.2. This method uses acid. Waste acid is hazardous to the sewer system and to the environment. All acid waste must be neutralized to a pH of 2.5-12 prior to disposal down the drain. The neutralization step is considered hazardous waste treatment and must be documented on the treatment by generator record. See the CAS EH&S Manual for details.

18. TRAINING

18.1. Training outline

18.1.1. Review literature (see references section). Read and understand the SOP. Also review the applicable MSDS for all reagents and standards used. Following these reviews, observe the procedure as performed by an experienced analyst at least three times.

18.1.2. The next training step is to assist in the procedure under the guidance of an experienced analyst. During this period, the analyst is expected to transition from a role of assisting, to performing the procedure with minimal oversight from an experienced analyst.

18.1.3. Perform initial precision and recovery (IPR) study as described above for water samples. Summaries of the IPR are reviewed and signed by the supervisor. Copies may be forwarded to the employee's training file. For applicable tests, IPR studies should be performed in order to be equivalent to NELAC's Initial Demonstration of Capability.

18.2. Training is documented following the SOP *for Documentation of Training* (ADM-TRANDOC).

NOTE: When the analyst training is documented by the supervisor on internal training documentation forms, the supervisor is acknowledging that the analyst has read and understands this SOP and that adequate training has been given to the analyst to competently perform the analysis independently.

19. METHOD MODIFICATIONS

19.1. There are no known differences between the reference method and this procedure

20. REFERENCES

20.1. USEPA, Test Methods for Evaluating Solid Waste, SW-846, 3rd Edition, Final Update II, Method 7471A, September 1994.

20.2. USEPA, Test Methods for Evaluating Solid Waste, SW-846, 3rd Edition, Update IV, Method 7471B, Revision 2, February 2007.

20.3. DoD Quality Systems Manual for Environmental Laboratories Version 4.1 4/22/2009.

21. CHANGES SINCE THE LAST REVISION

21.1. Sec 1.1 and 19 added method reference 7471B
21.2. Sec 1.2 removed PQL and updated MDL
21.3. Sec 6.1 and 6.3 are new
21.4. Sec 8.1 is new
21.5. Sec 9.1 is new
21.6. Sec 12.2.1 updated sop reference name
21.7. Sec 11.3 an d 12.3 updated CRA limits
21.8. Sec 12.5.2 updated LCS limits
21.9. Sec 12.5.3 updated % RPD criterion
21.10. Sec 12.5.4 updated post spike recovery limits
21.11. Sec 14 updated
21.12. Sec 19 is new

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ATTACHMENTS

Instrument Parameters
Benchsheets

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COLUMBIA ANALYTICAL SERVICES, INC. PRINTOUT WITH:_____

ANALYTICAL WORKSHEET

Method: (Circle One) 7470A 7471B 245.1	Service Request # :
Analysis For: Hg	

DATA

Pos.	SAMPLE NUMBER	Initial Sample (g) or (mL)	Initial Dilution (mL)	Dilution Factor	(µg/L) Measured	Sample Actual (mg/kg)	Sample Actual (µg/L)
1	Cal. Blk.	0.00	50	~			0.00
2	Std 0.2	*0.1	50	~			0.20
3	Std 0.5	*0.25	50	~			0.50
4	Std 1.0	*0.5	50	~			1.00
5	Std 5.0	*2.5	50	~			5.00
6	Std 10.0	*5.0	50	~			10.00
7		~	~	~			0%
8		~	~	~			< 0.2
9		~	~	~			0%
10		~	~	~			0%
11		~	~	~			< 0.2
12			50	~		#DIV/0!	
13			50	1/10		#DIV/0!	#DIV/0!
14			50	~		#DIV/0!	
15			50	~			0%
16			50	~		#DIV/0!	
17			50	~		#DIV/0!	#DIV/0!
18			50	~		#DIV/0!	
19			50	~		#DIV/0!	
20			50	~		#DIV/0!	
21			50	~		#DIV/0!	
22		~	~	~			0%
23		~	~	~			< 0.2
24			50	~		#DIV/0!	
25			50	~		#DIV/0!	

Comments: Reporting Levels: Cal. Inter. Std*_(100ppb)_____

Soil/Tissue Spike Level: 2nd Source Inter Std**_(1ppm)_____

Post Spike Level: x @ 5 µg/L

Method	Spike Level	MRL	LCS Limit	MS Limit	RPD
7470A Water	1.0 µg/L	0.2 µg/L	83-117%	80-120%	20%
245.1 Water	1.0 µg/L	0.2 µg/L	85-115%	70-130%	20%
7470A TCLP	5.0 µg/L	1.0 µg/L	85-115%	75-125%	20%
7471A Soil LCSS	6.80 mg/kg	0.02 mg/kg	72-128%	80-120%	30%
7471A Tissue Tort	0.27 mg/kg	0.02 mg/kg	63-130%	80-120%	30%

Analyst:	Date:	Page Number: 1

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Analytical Services™ Preparation Information Benchsheet

Prep Run: 141213 **Prep Workflow**: HgDigAq **Status**: Draft **Prep Date**: 09/02/2011 11:02
Team: Metals **Prep Method**: Method **Current Step**: Digestion **Due Date**: 09/05/2011
Analyst: MBEVANS **Rush/NPDES**: RUSH **Hold Date**: 09/20/2011

Lab Code	Client ID	Bottle #	Initial Amt	Final Volume	Spike Amt	Spike ID	TestNo List	Comments
KQ1109274-01	Method Blank						Hg D, Hg T	
KQ1109274-02	Lab Control Sample						Hg D, Hg T	
K1107897-001	PAG Pond	.04					Hg D	
K1107897-001	PAG Pond	.03					Hg T	
K1107897-001: KQ1109274-03	Duplicate	.04					Hg T	
K1107897-002	WQ 2	.04					Hg D	
K1107897-002	WQ 2	.03					Hg T	
K1107897-003	WQ 14	.04					Hg D	
K1107897-003	WQ 14	.03					Hg T	
K1107897-004	WQ 20	.04					Hg D	
K1107897-004	WQ 20	.03					Hg T	
K1107897-004: KQ1109274-04	Matrix Spike	.03					Hg T	
K1107980-001	WROU-MT3011	.01					Hg T	
K1107980-001: KQ1109274-05	Matrix Spike	.01					Hg T	
K1107980-001: KQ1109274-06	Duplicate Matrix Spike	.01					Hg T	
K1107980-019	WROU-SL3041	.01					Hg T	
K1107980-020	WROU-VG3040	.01					Hg T	
K1107980-021	WROU-VG3041	.01					Hg T	
K1108016-009	WROU-VG3042	.01					Hg T	
K1108016-010	WROU-VG3043	.01					Hg T	
K1108023-016	WROU-MT3012	.01					Hg T	
K1108023-017	WROU-MT3013	.01					Hg T	
K1108023-018	WROU-MT3014	.01					Hg T	

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K1108023-019	WROU-MT3015	.01					Hg T	

20 Total Samples consisting of 14 Client Samples, 4 Client QC Samples, 2 Batch QC Samples associated with the current Prep Run.

Spiking Solutions

Preparation Materials

Step	Name	ID
Digestion	K-MET K2S2O8 Hg	20924
Digestion	K-MET 50ml Centrifuge Tube	22573
Digestion	K-MET NH2OH-HCl Hg	27120
Digestion	K-MET SnCl Hg	30926

Step	Name	ID
Digestion	K-MET H2SO4 Hg	32787
Digestion	K-MET NACl Hg	32790
Digestion	K-MET KMnO4 Hg	32812
Digestion	K-MET HNO3 Hg	32853

Preparation Hardware / Equipment

Step	Name	Property	Value
Digestion	K-HotBlock-02	Temperature	_____ deg C

Preparation Steps

Step	Started	Finished	By	Assisted By	Training?	Comments
Digestion						

Comments

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STANDARD OPERATING PROCEDURE

for

DETERMINATION OF METALS AND TRACE ELEMENTS BY INDUCTIVELY COUPLED PLASMA ATOMIC EMISSION SPECTROMETRY (ICP)

SOP No.: MET-ICP

Revision 23
Effective Date: October 28, 2011

Approved by: _____ Supervisor — Date: 9/28/11

_____ QA Manager — Date: 9/29/11

_____ Laboratory Manager — Date: 9/29/11

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DOCUMENT CONTROL	
NUMBER:	
Initials:	Date:

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DETERMINATION OF METALS AND TRACE ELEMENTS BY INDUCTIVELY COUPLED PLASMA ATOMIC EMISSION SPECTROMETRY (ICP)

1 SCOPE AND APPLICATION

1.1 This procedure describes the steps taken for the analysis of soil, sludge surface water and drinking water digestates using EPA methods 6010C, 200.7, and CLP ILM04.0 for a variety of elements. The digested samples and QC standards are all diluted in a similar acid matrix. A procedure is also given for calculation of hardness by Standard Methods 2340B.

1.2 The Method Reporting Limits (MRLs) for common elements are listed in Table 1. Equivalent nomenclature for MRL includes Estimated Quantitation Limit (EQL). Therefore, MRL=EQL. The reported MRL may be adjusted if required for specific project requirements, however, the capability of achieving other reported MRLs must be demonstrated. The Method Detection Limits (MDLs) that have been achieved are listed in Table 1. The MDL and MRL may change as annual studies are performed.

1.3 In cases where there is a project-specific quality assurance plan (QAPP), the project manager identifies and communicates the QAPP-specific requirements to the laboratory. In general, project specific QAPP's supersede method specified requirements. An example of this are projects falling under DoD ELAP or project which require older versions of EPA methods (i.e. 6010B). QC requirements defined in the SOP *Department of Defense Projects – Laboratory Practices and Project Management (ADM-DOD)* may supersede the requirements defined in this SOP.

2 METHOD SUMMARY

2.1 A representative aliquot of sample is prepared as described in the applicable digestion SOP. The digestate is analyzed for the elements of interest using ICP spectrometry. The instrument measures characteristic emission spectra by optical spectrometry. The intensity of emission lines are monitored.

2.2 Final results are calculated using the digestion information and the results from the ICP analysis. Data is reported using standard CAS procedures and formats, or following project specific reporting specifications.

2.3 Deviations from the reference method(s): This SOP contains no deviations from the reference methods.

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3 DEFINITIONS

3.1 **Analysis Sequence** - Samples are analyzed in a set referred to as an analysis sequence. The sequence begins with instrument calibration followed by sample digestates interspersed with calibration standards.

3.2 **Independent Calibration Verification** (ICV) - ICV solutions are made from stock solutions different from the stock used to prepare calibration standards and are used to verify the validity of the standardization.

3.3 **Laboratory Control Sample** (LCS): A laboratory blank that has been fortified with target analyte and used to determine that the analysis is in control. For solids, a reference material may be used unless prohibited by project protocols.

3.4 **Matrix Spike** (MS) - In the matrix spike analysis, predetermined quantities of standard solutions of certain analytes are added to a sample matrix prior to sample digestion and analysis. The purpose of the matrix spike is to evaluate the effects of the sample matrix on the methods used for the analyses. Percent recoveries are calculated for each of the analytes detected.

3.5 **Matrix Spike Duplicate** (MSD) - In the matrix spike duplicate analysis, predetermined quantities of standard solutions of certain analytes are added to a sample matrix prior to sample digestion and analysis. The purpose of the matrix spike duplicate is to evaluate the effects of the sample matrix on the methods used for the analyses. Percent recoveries are calculated for each of the analytes detected. The relative percent difference between the matrix spikes is calculated and used to assess analytical precision.

3.6 **Duplicate Sample** (DUP) - A laboratory duplicate is a separate field sample aliquot that is processed in an identical manner as the sample proper. The relative percent difference between the samples is calculated and used to assess analytical precision.

3.7 **Method Blank** - The method blank is an artificial sample designed to monitor introduction of artifacts into the process. The method blank is carried through the entire analytical procedure.

3.8 **Continuing Calibration Verification Standard (CCV)** - A standard analyzed at specified intervals and used to verify the ongoing validity of the instrument calibration.

3.9 **Instrument Blank (CCB)** - The instrument blank (also called continuing calibration blank) is a volume of blank reagent of composition identical to the digestates. The purpose of the CCB is to determine the levels of contamination associated with the instrumental analysis.

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3.10 **Laboratory fortified Blank (LFB)**- A laboratory blank that has been fortified with target analyte at the method reporting limit and used to determine if the laboratory can detect contaminants at the method reporting limit.

4 INTERFERENCES

4.1 Interferences from contaminated reagents must be eliminated. The purity of acids must be established by the laboratory as being high enough to eliminate the introduction of contamination above the MRL (or above ½ the RL for DoD work).

4.2 Background emission and stray light can be compensated by background correction.

4.3 Spectral overlaps resulting in interelement contributions can be corrected for by using interelement correction factors. Interelement correction factors are established for each instrument and are maintained by the analyst at the workstation.

5 SAFETY

5.1 Chemicals, reagents and standards must be handled as described in the CAS safety policies, approved methods and in MSDSs where available. Refer to the CAS Environmental, Health and Safety Manual and the appropriate MSDS prior to beginning this method.

5.2 Hydrochloric, Nitric and Hydrofluoric Acids are used in this method. These acids are extremely corrosive and care must be taken while handling them. A face shield should be used while pouring acids. Safety glasses, lab coat and gloves should be worn while working with the solutions.

5.3 High Voltage - The power unit supplies high voltage to the RF generator which is used to form the plasma. The unit should never be opened. Exposure to high voltage can cause injury or death.

5.4 UV Light -The plasma when lit is a very intense light, and must not be viewed with the naked eye. Protective lenses are in place on the instrument. Glasses with special protective lenses are available.

6 SAMPLE COLLECTION, CONTAINERS, PRESERVATION, AND STORAGE

6.1 Samples are prepared using methods 3005A, 3010A, 3050, or CLPILM04.0 (CAS SOPs MET-3005A, MET-3010A, MET-3050, and MET-DIG). Samples are received in the ICP lab as completed digestates. Samples are stored in 50 mL plastic centrifuge tubes, 100 mL digestion vessels or in 100 mL volumetric flasks.

6.2 Water samples analyzed by EPA method 200.7 are preserved after arrival at the laboratory. These samples are held for a minimum of 16 hours and the pH verified to be <2 prior to digestion.

6.3 Soil samples are diluted prior to instrumental analysis by a factor of 2. This allows the method to meet the required 1 g of sample to 200 mL dilution during digestion.

6.4 Following analysis, digestates are stored until two weeks after all results have been reviewed and then brought to 3< pH<10 and disposed of through the sewer system.

7 APPARATUS & EQUIPMENT

7.1 Inductively Coupled Plasma Atomic Emission Spectrometer

7.1.1 Thermo Jarrell Ash IRIS.
7.1.2 Thermo Scientific ICAP 6500

7.2 Concentric nebulizers.

7.3 Microflow nebulizer for ICAP 6500.

7.4 Torches and injector tips for each ICP.

7.5 Cyclonic spray chambers for each instrument.

7.6 Water coolers for each ICP (internal on the IRIS.)

7.7 Argon Humidifiers for the IRIS and ICAP 6500.

7.8 ESI SC4 DX Autosampler with Fast System for ICAP 6500.

7.9 Peristaltic Pumps for each Spectrometer.

7.10 ASX-520 autosamplers for the IRIS.

7.11 RF Generators for each ICP (internal on the IRIS and ICAP 6500).

7.12 Computer system interfaced to each ICP. A compatible Windows-based data system is used to acquire, store, and perform calculations on raw data.

8 STANDARDS, REAGENTS, & CONSUMABLE MATERIALS

8.1 Standards Preparation

8.1.1 Stock standard solutions may be purchased from a number of vendors. All reference standards, where possible, must be traceable to SI units or NIST certified reference materials. The preparation for all laboratory prepared reagents and solutions must be documented in a laboratory logbook. Refer to the SOP

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Reagent/Standards Login and Tracking (ADM-RTL) for the complete procedure and documentation requirements. Manufacturer's expiration dates are used to determine the viability of standards.

8.1.2 Calibration Standards

Calibration standards are prepared from commercially purchased single element 1000 ppm or 10,000 ppm stock standards as well as pre-mixed multi element stock standards. All standards are aliquoted using Class A volumetric pipettes, or calibrated fixed and adjustable volume autopipetters. All dilutions are made in Class A volumetric glassware.

The standard mixes for each ICP system vary based on the requirements of each instrument. The composition of the IRIS calibration standards are outlined in Table 3. The composition of the ICAP 6500 standards are outlined in Table 4.

8.1.3 Continuing Calibration Verification (CCV) Standards

CCV standards are analyzed at the midpoint of the calibration. These standards are produced by making a two-fold dilution of each calibration standard. The CCV standards are then run in sequence during the analytical run.

8.1.4 Initial Calibration Verification (ICV) Standards

The ICV working standards are produced by direct dilution of three certified mixed stock solutions (QCP-CICV1, QCP-CICV2, and QCP-CICV3) purchased from Inorganic Ventures or another qualified vendor and various single element stock solutions from sources different than the calibration standards. The composition of these standards is outlined in Table 5.

8.1.5 Interference Check Solutions (ICSA & ICSAB)

The ICSA and ICSAB working standards are produced by direct dilution of certified mixed stock solutions (CLPP-ICS-A and CLPP-ICS-B or equivalent.) Antimony is also added to the ICSAB solution from a 1000 ppm single element stock standard. The composition of these standards is outlined in Table 6.

8.1.6 CRI/Low Level Calibration Verification

The CRI, Low Level Initial Calibration Verification (LLICV), and Low Level Continuing Calibration Verification (LLCCV) are produced by diluting 1000 or 10000ppm single stock standards into a 100X intermediate standard and then diluted 1/100 to obtain the MRL level. Note: The level used is that of the normal MRL used for both instruments.

8.1.7 The solutions and materials used for the LCS and matrix spikes are described in the applicable digestion SOP.

8.1.8 Standard Log

The analyte, source, initial volume, final volume, final concentration and expiration date are recorded in a standard logbook kept in the ICP lab. The operator who prepares the standard must date and initial the entry in the standards logbook. The operator also places his initials and the date prepared on the standard container. In addition to working standards used in calibration, all other standards used in the analytical run such as ICVs, MRL standards, and other project or client specific standards shall be documented in the standard logbook.

8.2 High Purity Argon.

8.3 Capillary, rinse and peristaltic pump tubing.

8.4 17 x 100mm polypropylene test tubes.

9 PREVENTIVE MAINTENANCE

9.1 All maintenance activities are recorded in a maintenance logbook kept for each instrument. Pertinent information (serial numbers, instrument I.D., etc.) must be in the logbook. This includes the routine maintenance described in section 9. The entry in the log must include: date of event, the initials of who performed the work, and a reference to analytical control.

9.2 Torch, nebulizer, and spray chambers are cleaned as required. All instrument filters are vacuumed monthly. Dirty ICP torches and mixing chambers are soaked in aqua regia overnight, rinsed and placed in a clean dry area. The conical nebulizer is back flushed with acid or DI water as needed. The microflow nebulizer is not back flushed. Use the obstruction removal kit with fused silica.

9.3 IRIS-specific procedures

9.3.1 Each time the nebulizer is changed, the instrument optimization procedure is performed. A 10 ppm As standard is aspirated and the intensity counts are maximized by adjusting the depth to which the nebulizer is inserted into the spray chamber.

10 RESPONSIBILITIES

10.1 It is the responsibility of the analyst to perform the analysis according to this SOP and to complete all documentation required for data review. Analysis and interpretation of the results are performed by personnel in the laboratory who have demonstrated the ability to generate acceptable results utilizing this SOP. This demonstration is in accordance with

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the training program of the laboratory. Final review and sign-off of the data is performed by the department supervisor/manager or designee.

10.2 It is the responsibility of the department supervisor/manager to document analyst training. Training and proficiency is documented in accordance with the SOP *ADM-TRANDOC*.

11 PROCEDURE

11.1 Operating Parameters

11.1.1 For the Thermo Jarrell Ash IRIS, the operating parameters are defined in the operating system *Method* file. Default operating parameters are given in file GALILEO. However, each unique set of operating parameters is saved as a new file and the analyst must select and use the correct *Method* file for the application. Refer to the method files on the workstation for a listing of parameters for each file. The interelement correction factors to be used are established for the IRIS and are saved on the workstation also. Since these parameters change with method and correction factor updates, and due to the large amount of hardcopy printout for listing these parameters, it is not practical to include the parameters in this SOP.

11.1.2 For the Thermo Scientific ICAP 6500, the operating parameters are defined in the *Method* file. Default operating parameters are given in *Tools/Options/New Method Parameters*. However, each unique set of operating parameters is saved as a new file and the analyst must select and use the correct *Method* file for the application. Refer to the method files on the workstation for a listing of parameters for each file. The interelement correction factors to be used are established for the ICAP 6500 and are saved on the workstation also. Since these parameters change with method and correction factor updates, and due to the large amount of hardcopy printout for listing these parameters, it is not practical to include the parameters in this SOP.

11.2 Calibration/Standardization

11.2.1 IRIS

11.2.1.1 Plasma is ignited and instrument is allowed to warm up for at least 30 minutes.

11.2.1.2 An internal standard is used for routine analyses on this instrument. Scandium is used as the internal standard. The internal standard solution is introduced into the analyzed solutions (standards, blanks, QC, samples, etc.) at 1ug/mL.

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11.2.1.3 Standardize by running a Blank and a High Standard for each element in the analytical method. Analyst will initial and date the first page of the standardization.

11.2.1.4 The Cu/Mn ratio is calculated from Standard A. Ratio is to remain within 20% of the value when IECs were determined. This data is recorded in the instrument logbook.

11.2.2 ICAP 6500

11.2.2.1 Plasma is ignited and instrument is allowed to warm up for at least 30 minutes.

11.2.2.2 An internal standard is used for routine analyses on this instrument. Yttrium and Indium are used as internal standards. The internal standard solution is introduced into the analyzed solutions (standards, blanks, QC, samples, etc.) at 0.8 ug/mL for Y, and 1.6 ug/mL for In.

11.2.2.3 Run a peak check standard and adjust peaks as needed.

11.2.2.4 Standardize by running a Blank and a High Standard for each element in the analytical method. Analyst will initial and date the first page of the standardization.

11.2.2.5 The Cu/Mn ratio is calculated from Standard A using Mn2576. Ratio is to remain within 20% of the value when IECs were determined. This data is recorded in the instrument logbook.

11.2.3 Standardization is completed by analyzing an ICV for each analyte to be determined. For method 200.7 the result must be within ± 5% of the true value. For method 6010B/C the result must be within ± 10% of the true value. If the ICV fails when running method 6010C, either the calibration standards or the ICV must be prepared fresh and the instrument re-standardized. If the ICV fails when running methods 200.7 and 6010B only re-standardization is necessary.

11.2.4 Method 6010C also requires a LLICV be analyzed at the MRL level. The result must be within ± 30% of the true value. The LLICV need not be made up with stock standards different than those of the calibration standards. A LLICV is not necessary when running methods 200.7 and 6010B.

11.3 Analytical Run

11.3.1 Following standardization and ICV analysis, the remainder of the run is determined by what analytical method is being performed. These are listed below.

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11.3.1.1 CLP ILM04.0: ICB, CCV, CCB, CRI, ICSA, ICSAB, CCV, CCB, routine samples. The CRI, ICSA, and ICSAB will be analyzed every 20 samples. They will be labeled with an F indicating Final. Each set will be numbered in increasing order, i.e. ICSAF1, ICSAF2.

11.3.1.2 Methods 200.7 and 6010B/C: ICB, CCV, CCB, CRI, ICSA, ICSAB, routine samples.

11.3.2 Evaluate the initial QC using the following criteria:

11.3.2.1 For methods 200.7 and 6010B/C, the following criteria apply:

- The ICB and CCB results are evaluated using method specified requirements. The following guidelines should also be used to determine acceptability:

- For 200.7, the result should be less than 3 times the standard deviation of the mean background signal.

- For method 6010B, the result should be less than the Method Detection Limit (MDL). In cases where the associated sample results are being reported to the Method Reporting Limit (MRL) the result may be greater than the MDL if the result does not adversely impact data quality.

- For method 6010C, the result should be less than the Lower Limit of Quantitation (LOQ).

- Where project specifications allow, the result may be over the MDL if the result does not adversely impact data quality.

- The CCV immediately following standardization must verify within ± 10% of the true values with a relative standard deviation of <5% from 2 replicate integrations for methods 6010B/C. For 200.7, the first CCV must verify within ± 5% with a RSD of <3% from 4 replicates. Calculate %RSD as follows:

$$\%RSD = \frac{StdDev_{CCV}}{Average_{CCV}} x\,100$$

where: $StdDev_{ccv}$ = Standard deviation of the replicate integrations
$Average_{ccv}$ = Average of the replicate CCV integrations

- The CRI is a low level standard with concentrations at the RL. For DoD projects, the CRI standard concentrations will be equal to the project RLs and results must verify within 20% of the true value. For method 6010C the CRI results should be within 30% of the true value. For 200.7 and 6010B the CRI results should be greater than the MDL and less than 2X the MRL. For method 6010C, the CRI may be run as the LICV/CRI and the results should be within 30% of the true value.

- The ICSA is run to check the validity of the Interelement Correction Factors (IECs).

Note: DoD QSM requires this to be run at the beginning of each analytical run.

- The ICSAB must be within 20% of the expected value for the CLPP-ICS-B elements and Sb.

11.3.2.2 The ICV, LLICV, ICB, CCV, CCB, CRI, and ICSAB must meet the criteria listed. Reanalyze any elements that fail.

11.3.2.3 For CLP, refer to SOW ILM04.0 for acceptance criteria.

11.3.3 Continuing Calibration Verification

11.3.3.1 CCVs are analyzed after every 10 samples and at the end of the analytical run. They must verify within ±10% of the expected value with a RSD of <10%.

11.3.3.2 CCBs are analyzed after every 10 samples and at the end of the analytical run. CCBs are evaluated as in section 11.3.2.1.

11.3.3.3 Method 6010C requires a LLCCV be analyzed at the end of each analysis batch. The LLCCV is at the MRL level and must verify within ± 30% of the true value. Reanalyze any elements to be reported at low levels that are bracketed by the LLCCV if the standard fails.

11.3.4 If the CCV, CCB or ICS solutions fail, reanalyze any elements to be reported.

12 QA/QC REQUIREMENTS

12.1 Initial Precision and Recovery Validation

The accuracy and precision of the procedure must be validated before analysis of samples begins, or whenever significant changes to the procedures have been made. To do this, four LCS aliquots are prepared and analyzed. The average percent recovery for each analyte must meet LCS criteria and the RSD< 30%.

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12.2 Method Detection Limits

12.2.1 A Method Detection Limit (MDL) study must be undertaken before analysis of samples can begin. To establish detection limits that are precise and accurate, the analyst must perform the following procedure. Spike a minimum of seven blank replicates at a level near or below the MRL. Follow the procedures in Section 11 to analyze the samples. Refer to the CAS SOP for *Performing Method Detection Limit Studies and Establishing Limits of Detection and Quantification (ADM-MDL.)*

12.2.2 Calculate the average concentration found (x) and the standard deviation of the concentrations for each analyte. Calculate the MDL for each analyte using the correct T value for the number of replicates. MDLs must be performed whenever there is a significant change in the background or instrument response.

12.2.3 A Limit of Detection (LOD) check must be performed after establishing the MDL and at least annually (quarterly if DoD) afterward. A blank is spiked with analytes at 1-4X the MDL and carried through the preparation and analytical procedure. The LOD is verified when the signal/noise ratio is > 3 for all analytes.

12.3 Limit of Quantitation Check(LOQ)/Lower Limit of Quantitation Check(LLQC)

For Method 6010C and drinking waters by method 200.7 a Lower Limit of Quantitation Check (LOQ/LLOQ) sample must be analyzed after establishing the MRL and at least annually (quarterly if DoD) afterward to demonstrate the desired detection capability. The LOQ/LLOQ sample is spiked at 1-2X the MRL and must be carried through the entire preparation and analytical procedure. Limits of quantitation are verified when all analytes are detected within 30% of their true value.

12.4 Linear Dynamic Range

The upper limit of the LDR must be established for each wavelength utilized. It must be determined from a linear calibration prepared in the normal manner using the established analytical operating procedure for the instrument. The LDR should be determined by analyzing at least three succeeding higher standard concentrations of the analyte until the observed analyte concentration is no more than 10% above or below the stated concentration of the standard. Determined LDRs must be documented and kept on file. The LDR which may be used for the analysis of samples should be judged by the analyst from the resulting data. Sample analyte concentrations that are greater than 90% of the determined upper LDR limit must be diluted and reanalyzed. The LDRs should be verified quarterly or whenever, in the judgment of the analyst, a change in analytical performance caused by either a change in instrument hardware or operating conditions would dictate they be redetermined.

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12.5 Instrument Detection Limit

On a quarterly basis, the instrument detection limits for all analytes are determined as per procedures outlined in ILM04.0 (Section E, paragraph 10, 12 resp.). IDLs are determined using blanks and this data is kept on file.

12.6 Interelement Correction Factors

Semi-annually, instrument interferences are calculated as per ILM04.0 (Section E, paragraph 11) and Method 6010B/C. During the course of routine work, other interferences may be found. They are verified by the operator during the analytical run and data is manually corrected. Copies of this data are kept on file.

12.7 Ongoing QC Samples required are described in the CAS-Kelso Quality Assurance Manual and in the SOP for *Sample Batches*. Additional QC Samples may be required in project specific quality assurance plans (QAPP). For example projects managed under the DoD ELAP must follow requirements defined in the DoD *Quality Systems Manual for Environmental Laboratories.* General QA requirements for DoD QSM are defined in the laboratory SOP, Department *of Defense Projects – Laboratory Practices and Project Management (ADM-DOD).* General QC Samples are:

12.7.1 Each sample preparation batch must have a method blank associated with it. The method blank result should be < MRL. If the method blank is found to be contaminated, it may be reported if the concentration in the associated samples is at least 20 times the amount found in the method blank for methods 200.7 and 6010B, otherwise redigest the batch. For Method 6010C, the method blank may be reported if the concentration in the associated samples is at least 10 times the amount found in the method blank.A contaminated method blank (MB) may also be reported if all of the associated samples are non-detect (ND).

Note: DoD QSM requires contamination in the MB be <1/2 the RL or < 1/10 any sample amount.

12.7.2 A Laboratory Control Sample (LCS) is digested one per batch, or per 20 samples. For method 200.7, the LCS recovery criteria is 85-115% for water samples. For method 6010B/C, the control limits are derived from lab data and are listed in Table 2. These limits should be no wider than 80-120%. For soil samples, the recovery must fall within the ranges specified for the reference material. For CLP, use the prescribed limits for the SOW in use. In all cases, project-specific QC limits may be required. If the LCS fails the acceptance criteria, redigest the batch of samples. For specifics on the preparation and composition of LCS samples refer to the appropriate digestion SOP.

12.7.3 A Duplicate sample is digested one per batch, or per 20 samples (i.e. 5%) for 6010B/C analysis, or per 10 samples (i.e. 10%) for 200.7 analyses. The default

criteria may be used if statistically generated criteria are broader or insufficient points are available for accurate statistical limits. Currently, statistically generated criteria are broader and the default is used for all elements but Manganese, for which the limit is 17% RPD. The RPD criteria are <30% for soil samples and <20% for water samples for methods 200.7 and 6010B. The RPD criteria is <20% for both soils and waters for method 6010C. Criteria are subject to change as statistical data are generated. If the RPD is outside acceptance limits, either redigest the sample batch or flag the data appropriately, depending on the physical nature of the samples (e.g. non-homogenous).

12.7.4 A Laboratory fortified Blank (LFB) at the MRL is digested and analyzed with every batch of drinking water samples (method 200.7). The default acceptance criteria of 50-150% are to be used until sufficient data points are acquired to calculate in-house control limits.

12.7.5 A Matrix Spike sample is digested one per batch, or per 20 samples (i.e. 5%) for 6010B/C analysis, or per 10 samples (i.e. 10%) for 200.7 analyses. Where specified by project requirements, a matrix spike duplicate may be required. Matrix spike recovery criteria for method 200.7 is 70-130% for both water and soil samples. For 6010B, the control limits are derived from lab data and are listed in Table 2. For 6010C, Table 2 is used unless the control limits are broader than the default criteria of 75-125% in which case the default criteria are used. For CLP, use the prescribed limits for the SOW in use. In all cases, project-specific QC limits may be required. If the recovery is outside acceptance limits, either redigest the sample batch or flag the data appropriately, depending on the physical nature of the samples (e.g. non-homogenous). If the sample concentration is >4x the spike level, no action is required and data is flagged accordingly. For specifics on the preparation and composition of matrix spike solutions refer to the appropriate digestion SOP.

12.7.6 Matrix Interference

12.7.6.1 When an analyst suspects that there may be any matrix interferences present, a 1:5 serial dilution test shall be performed. The dilution should be within ± 10% of the original result.

12.7.6.2 A 1:5 serial dilution shall be performed for all Tier III or IV deliverables.

12.7.6.3 If the analyte concentration is too low to permit a dilution test, then a post digestion spike may be performed. The recovery should be ± 20%.

12.7.6.4 For Method 6010C, lower tier batches that exhibit MS failure require a serial dilution test as well as a post digestion spike.

Note: DoD QSM recovery acceptance limits are 75-125%.

12.8 Additional QC measures include control charting and compiling of QC data for generation of control limits.

12.9 CLP analyses are performed as per the QA/QC guidelines in the most current CLP SOW.

13 DATA REDUCTION, REVIEW, AND REPORTING

13.1 Calculate sample results using the data system printouts and digestion information. The digestion and dilution information is entered into the data system. The data system then uses the calculations below to generate a sample result. The wavelengths used to quantify each metal are summarized in Table 7 for the IRIS and Table 8 for the ICAP6500.

Aqueous samples are reported in µg/L:

$$\mu g/L\,(Sample) = C^{*}\ x\ Digestion\,Dilution\,Factor\ x\ Post\,Digestion\,Dilution\,Factor \times 1000\,\mu g/mg$$

Solid samples are reported in mg/Kg:

$$mg/Kg\,(Sample) = C^{*}\ x\ Post\,Digestion\,Dilution\,Factor\ x\ \frac{Digestion\,Vol\,(ml)}{Sample\,wt\,(g)}\ x\ \frac{1L}{1000ml}\ x\ \frac{1000g}{1Kg}$$

C*= Concentration of analyte as measured at the instrument in mg/L.

13.2 If total hardness is to be reported, use Calcium and Magnesium results to calculate as follows. For reporting calcium hardness, use only the calcium portion of the equation.

$$Hardness, mg\ equivalent\ CaCO_3/L = 2.497\left[Ca, mg/L\right] + 4.118\left[Mg, mg/L\right]$$

13.3 A daily run log of all samples analyzed is maintained. All CLP data should be printed and stored after operator has checked for evenness of burns. A copy of this document will go with each package of Tier III or higher data run that day.

13.4 Data Review and Reporting

13.4.1 It is the analyst's responsibility to review analytical data to ensure that all quality control requirements have been met for each analytical run. Results for QC analyses are calculated and recorded as specified in section 12. The data is then placed in a work order file until complete. When the work order is complete, a report is generated. A final review is performed and the data is delivered to the project management department.

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14 CORRECTIVE ACTION

14.1 Refer to the SOP for *Corrective Action* for procedures for corrective action. Personnel at all levels and positions in the laboratory are to be alert to identifying problems and nonconformities when errors, deficiencies, or out-of-control situations are detected.

14.2 Handling out-of-control or unacceptable data

14.2.1 On-the-spot corrective actions that are routinely made by analysts and result in acceptable analyses should be documented as normal operating procedures, and no specific documentation need be made other than notations in laboratory maintenance logbooks, runlogs, for example.

14.2.2 Documentation of a nonconformity must be done using a Nonconformity and Corrective Action Report (NCAR) when:

- Corrective action is not taken or not possible
- Corrective action fails to correct an out-of-control problem on a laboratory QC or calibration analysis.
- Reanalysis corrects the nonconformity but is not a procedurally compliant analysis.

15 METHOD PERFORMANCE

This method was validated through single laboratory studies of accuracy and precision. Refer to the reference method for additional available method performance data.

15.1 The method detection limit (MDL) is established using the procedure described in the SOP for *Performing Method Detection Limit Studies and Establishing Limits of Detection and Quantification (ADM-MDL)*. Method Reporting Limits are established for this method based on MDL studies and as specified in the CAS Quality Assurance Manual.

16 POLLUTION PREVENTION

It is the laboratory's practice to minimize the amount of solvents and reagents used to perform this method wherever technically sound, feasibly possible and within method requirements. Standards are prepared in volumes consistent with the laboratory use in order to minimize the volume of expired standards to be disposed. The threat to the environment from reagents used in this method may be minimized when recycled or disposed of properly.

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17 WASTE MANAGEMENT

17.1 The laboratory will comply with all Federal, State and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions as specified in the CAS EH&S Manual.

17.2 This method uses acid. Waste acid is hazardous to the sewer system and to the environment. All acid waste must be neutralized to a pH of 3-10 prior to disposal down the drain. The neutralization step is considered hazardous waste treatment and must be documented on the treatment by generator record. See the CAS EH&S Manual for details.

18 TRAINING

18.1 Refer to the *SOP for Documentation of Training* for standard procedures.

18.2 Training outline

18.2.1 Review literature (see references section). Review the SOP. Also review the applicable MSDS for all reagents and standards used. Following these reviews, observe the procedure as performed by an experienced analyst at least three times.

18.2.2 The next training step is to assist in the procedure under the guidance of an experienced analyst for a period of approximately two weeks. During this period, the analyst is expected to transition from a role of assisting, to performing the procedure with minimal oversight from an experienced analyst.

18.2.3 Perform initial precision and recovery (IPR) study as described in Section 12.1 for water samples. Summaries of the IPR are reviewed and signed by the supervisor.

18.3 Training and proficiency is documented in accordance with the SOP ADM-TRANDOC.

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19 CHANGES SINCE THE LAST REVISION

19.1 Sec 1.2 removed PQL – outdated definition
19.2 Sec 8.1.1 updated to reflect current lab QA system requirements
19.3 7.2: Added Microflow nebulizer for ICAP 6500.
19.4 7.8: Added ESI SC4 DX autosampler with fast system for ICAP 6500.
19.5 Sec 9.1 updated to reflect current lab QA system requirements
19.6 Sec 9.2 Added maintenance procedure for the Microflow nebulizer.
19.7 11.2.2.2: Updated internal standards: y = 0.8 µg/mL; 1n =1.6 µg/mL
19.8 11.3.2.1: 6010- added use of the CRI for the LLCV/CRI and acceptance criteria.
19.9 12.7.1: Added reporting of a contaminated MB if associated samples are ND.
19.10 12.7.6: Revised section on matrix interference.
19.11 11.3.3.3: Removed requirement to run ICS solutions every 8 hours.
19.12 Table 3 Standard B Iris ICP-OES, Mn aliquot 10 mL; final concentration 10 ppm.
19.13 Table 5 ICVB Solution updates for Li -2ppm; Fe and Mn -10ppm;
19.14 Table 8 update to include alternate frequency wavelengths for Cadmium, Cobalt, Copper Phosphorous, Nickel and Zinc.
19.15 Corporate SOP name change; ADM-DATANTRY *Making Entries into Logbooks and onto Analytical Records.*
19.16 Tables 1 & 2 updated

20 REFERENCES

20.1 USEPA, Contract Laboratory Program, SOW #ILM04.0

20.2 Thermo Jarrell Ash ICAP61 Manual

20.3 USEPA, Test Methods for Evaluating Solid Waste, SW-846, 3rd Edition, Update III, Method 6010B, Revision 2, December 1996.

20.4 USEPA, Test Methods for Evaluating Solid Waste, SW-846, 3rd Edition, Update III, Method 6010C, Revision 3, February 2007.

20.5 USEPA, Methods for Determination of Metals in Environmental Samples, Supplement I, EPA/600/R-94/111, Method 200.7, Revision 4.4, May 1994.

20.6 *Hardness by Calculation, Method 2340B*, Standard Methods for the Examination of Water and Wastewater, 20th ed., 1998.

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CAS/KELSO DATA QUALITY OBJECTIVES **Table 1 & 2**

METHOD	ANALYTE	CAS No.	MATRIX	MDLa	MRL	UNITS	Accuracy (LCS %Rec.)	Matrix Spike (%Rec.)	Precision (% RPD)
200.7	Aluminum	7429-90-5	Soil	6	10	mg/kg	41-158	70-130	30
200.7	Antimony	7440-36-0	Soil	3	10	mg/kg	50-150	70-130	30
200.7	Arsenic	7440-38-2	Soil	4	20	mg/kg	75-125	70-130	30
200.7	Barium	7440-39-3	Soil	0.3	2	mg/kg	81-119	70-130	30
200.7	Beryllium	7440-41-7	Soil	0.03	1	mg/kg	83-117	70-130	30
200.7	Boron	7440-42-8	Soil	0.4	10	mg/kg	67-133	70-130	30
200.7	Cadmium	7440-43-9	Soil	0.2	1	mg/kg	81-119	70-130	30
200.7	Calcium	7440-70-2	Soil	2	10	mg/kg	79-121	70-130	30
200.7	Chromium	7440-47-3	Soil	0.4	2	mg/kg	80-119	70-130	30
200.7	Cobalt	7440-48-4	Soil	0.3	2	mg/kg	82-118	70-130	30
200.7	Copper	7440-50-8	Soil	0.6	2	mg/kg	83-116	70-130	30
200.7	Iron	7439-89-6	Soil	0.7	4	mg/kg	50-149	70-130	30
200.7	Lead	7439-92-1	Soil	3	20	mg/kg	79-121	70-130	30
200.7	Lithium	7439-93-2	Soil	0.5	2	mg/kg	75-125	70-130	30
200.7	Magnesium	7439-95-4	Soil	0.3	4	mg/kg	73-127	70-130	30
200.7	Manganese	7439-96-5	Soil	0.04	2	mg/kg	81-119	70-130	30
200.7	Molybdenum	7439-98-7	Soil	0.5	2	mg/kg	75-125	70-130	30
200.7	Nickel	7440-02-0	Soil	0.5	4	mg/kg	81-118	70-130	30
200.7	Phosphorus	7723-14-0	Soil	3	40	mg/kg	75-125	70-130	30
200.7	Potassium	7440-09-7	Soil	20	80	mg/kg	73-126	70-130	30
200.7	Selenium	7782-49-2	Soil	4	20	mg/kg	75-125	70-130	30
200.7	Silver	7440-22-4	Soil	0.4	2	mg/kg	66-134	70-130	30
200.7	Sodium	7440-23-5	Soil	4	40	mg/kg	74-126	70-130	30
200.7	Strontium	7440-24-6	Soil	0.02	2	mg/kg	79-121	70-130	30
200.7	Thallium	7440-28-0	Soil	3	20	mg/kg	75-125	70-130	30
200.7	Tin	7440-31-5	Soil	2	10	mg/kg	75-124	70-130	30
200.7	Titanium	7440-32-6	Soil	0.5	2	mg/kg	75-125	70-130	30
200.7	Vanadium	7440-62-2	Soil	0.4	2	mg/kg	79-121	70-130	30
200.7	Zinc	7440-66-6	Soil	0.3	2	mg/kg	73-121	70-130	30

a Method Detection Limits are subject to change as new MDL studies are completed.

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CAS/KELSO DATA QUALITY OBJECTIVES

METHOD	ANALYTE	CAS No.	MATRIX	MDLa	MRL	LODb	LOQc	UNITS	Accuracy (LCS %Rec.)	Matrix Spike (%Rec.)	Precision (% RPD)
200.7	Aluminum	7429-90-5	Water	30	50	28	50	ug/L	85-115	70-130	20
200.7	Antimony	7440-36-0	Water	20	50	30	50	ug/L	85-115	70-130	20
200.7	Arsenic	7440-38-2	Water	20	100	30	100	ug/L	85-115	70-130	20
200.7	Barium	7440-39-3	Water	1	5	1.5	5	ug/L	85-115	70-130	20
200.7	Beryllium	7440-41-7	Water	0.2	5	0.3	5	ug/L	85-115	70-130	20
200.7	Boron	7440-42-8	Water	3	50		50	ug/L	85-115	70-130	20
200.7	Cadmium	7440-43-9	Water	0.8	5	2	5	ug/L	85-115	70-130	20
200.7	Calcium	7440-70-2	Water	10	50	25	50	ug/L	85-115	70-130	20
200.7	Chromium	7440-47-3	Water	3	5	2.5	5	ug/L	85-115	70-130	20
200.7	Cobalt	7440-48-4	Water	2	10	2.5	10	ug/L	85-115	70-130	20
200.7	Copper	7440-50-8	Water	5	10	4.5	10	ug/L	85-115	70-130	20
200.7	Iron	7439-89-6	Water	2	20	4	20	ug/L	85-115	70-130	20
200.7	Lead	7439-92-1	Water	8	50	30	50	ug/L	85-115	70-130	20
200.7	Lithium	7439-93-2	Water	2	10	3.5	10	ug/L	85-115	70-130	20
200.7	Magnesium	7439-95-4	Water	0.7	20	0.5	20	ug/L	85-115	70-130	20
200.7	Manganese	7439-96-5	Water	0.4	5	0.5	5	ug/L	85-115	70-130	20
200.7	Molybdenum	7439-98-7	Water	3	10	5	10	ug/L	85-115	70-130	20
200.7	Nickel	7440-02-0	Water	2	20	4	20	ug/L	85-115	70-130	20
200.7	Phosphorus	7723-14-0	Water	30	200	40	200	ug/L	85-115	70-130	20
200.7	Potassium	7440-09-7	Water	50	400	80	400	ug/L	85-115	70-130	20
200.7	Selenium	7782-49-2	Water	30	100	40	100	ug/L	85-115	70-130	20
200.7	Silicon	7440-21-3	Water	10	400		400	ug/L	85-115	70-130	20
200.7	Silver	7440-22-4	Water	5	10	5	10	ug/L	85-115	70-130	20
200.7	Sodium	7440-23-5	Water	30	200	20	200	ug/L	85-115	70-130	20
200.7	Strontium	7440-24-6	Water	0.06	10			ug/L	85-115	70-130	20
200.7	Thallium	7440-28-0	Water	30	100	60	100	ug/L	85-115	70-130	20
200.7	Tin	7440-31-5	Water	20	50	30	50	ug/L	85-115	70-130	20
200.7	Titanium	7440-32-6	Water	5	10	5	10	ug/L	85-115	70-130	20
200.7	Vanadium	7440-62-2	Water	5	10	5	10	ug/L	85-115	70-130	20
200.7	Zinc	7440-66-6	Water	2	10	2.5	10	ug/L	85-115	70-130	20

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CAS/KELSO DATA QUALITY OBJECTIVES

METHOD	ANALYTE	CAS No.	MATRIX	MDLa	MRL	LODb	LOQc	UNITS	Accuracy (LCS %Rec.)	Matrix Spike (%Rec.)	Precision (% RPD)
6010C	Aluminum	7429-90-5	Water	40	50	40	50	ug/L	92-112	75-125	20
6010C	Antimony	7440-36-0	Water	10	50	30	50	ug/L	90-113	75-125	20
6010C	Arsenic	7440-38-2	Water	20	100	30	100	ug/L	90-112	75-125	20
6010C	Barium	7440-39-3	Water	0.5	5	1.5	5	ug/L	91-113	75-125	20
6010C	Beryllium	7440-41-7	Water	0.2	5	0.3	5	ug/L	91-113	75-125	20
6010C	Boron	7440-42-8	Water	2	50	4	50	ug/L	91-112	75-125	20
6010C	Cadmium	7440-43-9	Water	0.9	5	2	5	ug/L	93-113	75-125	20
6010C	Calcium	7440-70-2	Water	9	50	25	50	ug/L	85-116	75-125	20
6010C	Chromium	7440-47-3	Water	2	5	5	5	ug/L	93-114	75-125	20
6010C	Cobalt	7440-48-4	Water	2	10	2.5	10	ug/L	93-114	75-125	20
6010C	Copper	7440-50-8	Water	5	10	9	10	ug/L	91-111	75-125	20
6010C	Iron	7439-89-6	Water	3	20	4	20	ug/L	92-111	75-125	20
6010C	Lead	7439-92-1	Water	8	50	30	50	ug/L	92-113	75-125	20
6010C	Lithium	7439-93-2	Water	2	10	3.5	10	ug/L	80-120	75-125	20
6010C	Magnesium	7439-95-4	Water	0.4	20	0.5	20	ug/L	86-115	75-125	20
6010C	Manganese	7439-96-5	Water	0.7	5	0.5	5	ug/L	92-112	75-125	20
6010C	Molybdenum	7439-98-7	Water	2	10	5	10	ug/L	92-113	75-125	20
6010C	Nickel	7440-02-0	Water	3	20	4	20	ug/L	91-118	75-125	20
6010C	Phosphorus	7723-14-0	Water	60	200	40	200	ug/L	80-120	75-125	20
6010C	Potassium	7440-09-7	Water	40	400	80	400	ug/L	89-114	75-125	20
6010C	Selenium	7782-49-2	Water	20	100	40	100	ug/L	88-113	75-125	20
6010C	Silicon	7440-21-3	Water	6	400	8	400	ug/L	80-120	75-125	20
6010C	Silver	7440-22-4	Water	5	10	10	10	ug/L	93-110	75-125	20
6010C	Sodium	7440-23-5	Water	20	200	20	200	ug/L	80-120	75-125	20
6010C	Strontium	7440-24-6	Water	0.9	10	0.25	10	ug/L	80-120	75-125	20
6010C	Thallium	7440-28-0	Water	30	100	60	100	ug/L	80-120	75-125	20
6010C	Tin	7440-31-5	Water	9	50	30	50	ug/L	80-120	75-125	20
6010C	Titanium	7440-32-6	Water	4	10	10	10	ug/L	80-120	75-125	20
6010C	Vanadium	7440-62-2	Water	6	10	6.25	10	ug/L	92-111	75-125	20
6010C	Zinc	7440-66-6	Water	2	10	2.5	10	ug/L	92-112	75-125	20

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METHOD	ANALYTE	CAS No.	MATRIX	MDLa	MRL	LODb	LOQc	UNITS	Accuracy (LCS %Rec.)	Matrix Spike (%Rec.)	Precision (% RPD)
6010C	Aluminum	7429-90-5	Soil	6	10	10	10	mg/kg	41-158	75-125	20
6010C	Antimony	7440-36-0	Soil	3	10	6	10	mg/kg	50-150	75-125	20
6010C	Arsenic	7440-38-2	Soil	4	20	8	20	mg/kg	78-122	75-125	20
6010C	Barium	7440-39-3	Soil	0.08	2	0.16	2	mg/kg	81-119	75-125	20
6010C	Beryllium	7440-41-7	Soil	0.02	1	0.04	1	mg/kg	83-117	75-125	20
6010C	Boron	7440-42-8	Soil	0.4	10	0.8	10	mg/kg	67-133	75-125	20
6010C	Cadmium	7440-43-9	Soil	0.3	1	0.6	1	mg/kg	81-119	75-125	20
6010C	Calcium	7440-70-2	Soil	2	10	4	10	mg/kg	79-121	75-125	20
6010C	Chromium	7440-47-3	Soil	0.5	2	1	2	mg/kg	80-119	75-125	20
6010C	Cobalt	7440-48-4	Soil	0.4	2	0.8	2	mg/kg	82-118	75-125	20
6010C	Copper	7440-50-8	Soil	0.7	2	1.4	2	mg/kg	83-116	75-125	20
6010C	Iron	7439-89-6	Soil	0.7	4	1.4	4	mg/kg	50-149	75-125	20
6010C	Lead	7439-92-1	Soil	3	20	6	20	mg/kg	79-121	75-125	20
6010C	Lithium	7439-93-2	Soil	0.5	2	0.8	2	mg/kg	75-125	75-125	20
6010C	Magnesium	7439-95-4	Soil	0.08	4	0.16	4	mg/kg	73-127	75-125	20
6010C	Manganese	7439-96-5	Soil	0.04	2	0.08	2	mg/kg	81-119	75-125	20
6010C	Molybdenum	7439-98-7	Soil	0.7	2	1.4	4.5	mg/kg	75-125	75-125	20
6010C	Nickel	7440-02-0	Soil	0.6	4	1.2	4	mg/kg	81-118	75-125	20
6010C	Phosphorus	7723-14-0	Soil	4	40	8	40	mg/kg	75-125	75-125	20
6010C	Potassium	7440-09-7	Soil	20	80	40	80	mg/kg	73-126	75-125	20
6010C	Selenium	7782-49-2	Soil	5	20	10	20	mg/kg	80-120	75-125	20
6010C	Silver	7440-22-4	Soil	2	2	4	4	mg/kg	66-134	75-125	20
6010C	Sodium	7440-23-5	Soil	4	40	8	40	mg/kg	74-126	75-125	20
6010C	Strontium	7440-24-6	Soil	0.02	2	0.04	2	mg/kg	79-121	75-125	20
6010C	Thallium	7440-28-0	Soil	7	20	14	20	mg/kg	79-120	75-125	20
6010C	Tin	7440-31-5	Soil	4	10	8	10	mg/kg	75-124	75-125	20
6010C	Titanium	7440-32-6	Soil	0.8	2	1.6	2	mg/kg	75-125	75-125	20
6010C	Vanadium	7440-62-2	Soil	2	2	2	2	mg/kg	79-121	75-125	20
6010C	Zinc	7440-66-6	Soil	0.3	2	0.6	2	mg/kg	73-121	75-125	20

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CAS/KELSO DATA QUALITY OBJECTIVES

METHOD	ANALYTE	CAS No.	MATRIX	MDLa	MRL	LODb	LOQc	UNITS	Accuracy (LCS %Rec.)	Matrix Spike (%Rec.)	Precision (% RPD)
6010C LL	Aluminum	7429-90-5	Soil	0.4	1	0.8	2	mg/kg	41-158	75-125	20
6010C LL	Antimony	7440-36-0	Soil	0.5	2	1	3	mg/kg	50-150	75-125	20
6010C LL	Arsenic	7440-38-2	Soil	0.9	2	1.8	5	mg/kg	78-122	75-125	20
6010C LL	Barium	7440-39-3	Soil	0.06	0.5	0.12	0.5	mg/kg	81-119	75-125	20
6010C LL	Beryllium	7440-41-7	Soil	0.03	0.1	0.06	0.2	mg/kg	83-117	75-125	20
6010C LL	Boron	7440-42-8	Soil	0.4	2	1.6	10	mg/kg	67-133	75-125	20
6010C LL	Cadmium	7440-43-9	Soil	0.03	0.1	0.06	0.18	mg/kg	81-119	75-125	20
6010C LL	Calcium	7440-70-2	Soil	0.6	2	1.2	3.6	mg/kg	79-121	75-125	20
6010C LL	Chromium	7440-47-3	Soil	0.2	0.5	0.4	1.2	mg/kg	80-119	75-125	20
6010C LL	Cobalt	7440-48-4	Soil	0.2	0.5	0.4	1.2	mg/kg	82-118	75-125	20
6010C LL	Copper	7440-50-8	Soil	0.3	0.6	0.6	1.8	mg/kg	83-116	75-125	20
6010C LL	Iron	7439-89-6	Soil	0.7	2	1.4	4.2	mg/kg	50-149	75-125	20
6010C LL	Lead	7439-92-1	Soil	0.4	2	0.8	2	mg/kg	79-121	75-125	20
6010C LL	Lithium	7439-93-2	Soil	0.5	2	1	3	mg/kg	80-120	75-125	20
6010C LL	Magnesium	7439-95-4	Soil	0.06	0.5	0.12	0.5	mg/kg	73-127	75-125	20
6010C LL	Manganese	7439-96-5	Soil	0.02	0.2	0.04	0.2	mg/kg	81-119	75-125	20
6010C LL	Molybdenum	7439-98-7	Soil	0.08	0.4	0.06	0.4	mg/kg	75-125	75-125	20
6010C LL	Nickel	7440-02-0	Soil	0.07	0.4	0.14	0.4	mg/kg	81-118	75-125	20
6010C LL	Phosphorus	7723-14-0	Soil	3	6	6	40	mg/kg	80-120	75-125	20
6010C LL	Potassium	7440-09-7	Soil	20	60	40	120	mg/kg	73-126	75-125	20
6010C LL	Selenium	7782-49-2	Soil	0.7	4	1.4	4.2	mg/kg	80-120	75-125	20
6010C LL	Silver	7440-22-4	Soil	0.2	0.5	0.4	1.2	mg/kg	66-134	75-125	20
6010C LL	Sodium	7440-23-5	Soil	4	40	8	40	mg/kg	74-126	75-125	20
6010C LL	Strontium	7440-24-6	Soil	0.02	2	0.06	2	mg/kg	80-120	75-125	20
6010C LL	Thallium	7440-28-0	Soil	0.4	2	0.4	20	mg/kg	79-120	75-125	20
6010C LL	Tin	7440-31-5	Soil	0.7	10	1.6	10	mg/kg	75-124	75-125	20
6010C LL	Titanium	7440-32-6	Soil	0.05	0.2	0.16	2	mg/kg	80-120	75-125	20
6010C LL	Vanadium	7440-62-2	Soil	0.3	1	0.6	2	mg/kg	79-121	75-125	20
6010C LL	Zinc	7440-66-6	Soil	0.3	1	0.6	2	mg/kg	73-121	75-125	20

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CAS/KELSO DATA QUALITY OBJECTIVES

METHOD	ANALYTE	CAS No.	MATRIX	MDLa	MRL	LODb	LOQc	UNITS	Accuracy (LCS %Rec.)	Matrix Spike (%Rec.)	Precision (% RPD)
6010C LL	Aluminum	7429-90-5	Water	0.5	2	6	18	ug/L	92-112	75-125	20
6010C LL	Antimony	7440-36-0	Water	3	10	6	18	ug/L	90-113	75-125	20
6010C LL	Arsenic	7440-38-2	Water	4	10	8	24	ug/L	90-112	75-125	20
6010C LL	Barium	7440-39-3	Water	0.4	2	0.8	2.4	ug/L	91-113	75-125	20
6010C LL	Beryllium	7440-41-7	Water	0.09	0.2	0.18	0.6	ug/L	91-113	75-125	20
6010C LL	Boron	7440-42-8	Water	2	10	8	50	ug/L	91-112	75-125	20
6010C LL	Cadmium	7440-43-9	Water	0.3	0.5	0.6	1.8	ug/L	93-113	75-125	20
6010C LL	Calcium	7440-70-2	Water	2	4	20	50	ug/L	85-116	75-125	20
6010C LL	Chromium	7440-47-3	Water	0.4	2	0.8	2.4	ug/L	93-114	75-125	20
6010C LL	Cobalt	7440-48-4	Water	0.4	1	0.8	2.4	ug/L	93-114	75-125	20
6010C LL	Copper	7440-50-8	Water	2	2	4	12	ug/L	91-111	75-125	20
6010C LL	Iron	7439-89-6	Water	3	10	6	18	ug/L	92-111	75-125	20
6010C LL	Lead	7439-92-1	Water	4	10	8	24	ug/L	92-113	75-125	20
6010C LL	Lithium	7439-93-2	Water	2	10	4	12	ug/L	80-120	75-125	20
6010C LL	Magnesium	7439-95-4	Water	0.4	2	6	20	ug/L	86-115	75-125	20
6010C LL	Manganese	7439-96-5	Water	0.2	0.6	0.4	2.4	ug/L	92-112	75-125	20
6010C LL	Molybdenum	7439-98-7	Water	0.6	2	1.2	3.6	ug/L	92-113	75-125	20
6010C LL	Nickel	7440-02-0	Water	0.7	2	1.4	4.2	ug/L	91-118	75-125	20
6010C LL	Phosphorus	7723-14-0	Water	7	20	7	400	ug/L	80-120	75-125	20
6010C LL	Potassium	7440-09-7	Water	50	100	100	300	ug/L	89-114	75-125	20
6010C LL	Selenium	7782-49-2	Water	5	20	10	30	ug/L	88-113	75-125	20
6010C LL	Silicon	7440-21-3	Water	10	50	10	400	ug/L	80-120	75-125	20
6010C LL	Silver	7440-22-4	Water	0.7	2	1.4	4.2	ug/L	93-110	75-125	20
6010C LL	Sodium	7440-23-5	Water	70	200	140	420	ug/L	80-120	75-125	20
6010C LL	Strontium	7440-24-6	Water	0.07	0.2	0.07	10	ug/L	80-120	75-125	20
6010C LL	Thallium	7440-28-0	Water	2	10	6	18	ug/L	80-120	75-125	20
6010C LL	Tin	7440-31-5	Water	2	10	2	10	ug/L	80-120	75-125	20
6010C LL	Titanium	7440-32-6	Water	0.2	1	0.8	10	ug/L	80-120	75-125	20
6010C LL	Vanadium	7440-62-2	Water	1	2	2	6	ug/L	92-111	75-125	20
6010C LL	Zinc	7440-66-6	Water	0.7	2	1.4	4.2	ug/L	92-112	75-125	20

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CAS/KELSO DATA QUALITY OBJECTIVES

METHOD	ANALYTE	CAS No.	MATRIX	MDLa	MRL	UNITS	Accuracy (LCS %Rec.)	Matrix Spike (%Rec.)	Precision (% RPD)
6010C/PSEP	Aluminum	7429-90-5	Tissue	0.07	1	mg/kg	75-125	70-130	30
6010C/PSEP	Antimony	7440-36-0	Tissue	0.4	5	mg/kg	75-125	70-130	30
6010C/PSEP	Arsenic		Tissue	0.6	10	mg/kg	75-125	70-130	30
6010C/PSEP	Barium	7440-39-3	Tissue	0.04	0.5	mg/kg	75-125	70-130	30
6010C/PSEP	Beryllium		Tissue	0.02	0.5	mg/kg	75-125	70-130	30
6010C/PSEP	Boron	7440-42-8	Tissue	0.2	5	mg/kg	75-125	70-130	30
6010C/PSEP	Cadmium	7440-43-9	Tissue	3	5	mg/kg	75-125	70-130	30
6010C/PSEP	Calcium	7440-70-2	Tissue	2	5	mg/kg	75-125	70-130	30
6010C/PSEP	Chromium	7440-47-3	Tissue	0.08	0.2	mg/kg	75-125	70-130	30
6010C/PSEP	Cobalt		Tissue	0.05	1	mg/kg	75-125	70-130	30
6010C/PSEP	Copper	7440-50-8	Tissue	0.2	1	mg/kg	75-125	70-130	30
6010C/PSEP	Iron	7439-89-6	Tissue	0.4	2	mg/kg	75-125	70-130	30
6010C/PSEP	Lead		Tissue	0.2	5	mg/kg	75-125	70-130	30
6010C/PSEP	Lithium	7439-93-2	Tissue	0.3	0.5	mg/kg	75-125	70-130	30
6010C/PSEP	Magnesium	7439-95-4	Tissue	0.4	2	mg/kg	75-125	70-130	30
6010C/PSEP	Manganese	7439-96-5	Tissue	0.03	0.5	mg/kg	75-125	70-130	30
6010C/PSEP	Molybdenum	7439-98-7	Tissue	0.05	1	mg/kg	75-125	70-130	30
6010C/PSEP	Nickel	7440-02-0	Tissue	0.06	2	mg/kg	75-125	70-130	30
6010C/PSEP	Phosphorus	7723-14-0	Tissue	2	20	mg/kg	75-125	70-130	30
6010C/PSEP	Potassium	7440-09-7	Tissue	6	40	mg/kg	75-125	70-130	30
6010C/PSEP	Selenium	7782-49-2	Tissue	0.7	10	mg/kg	75-125	70-130	30
6010C/PSEP	Silver	7440-22-4	Tissue	0.1	1	mg/kg	75-125	70-130	30
6010C/PSEP	Sodium	7440-23-5	Tissue	4	20	mg/kg	75-125	70-130	30
6010C/PSEP	Strontium	7440-24-6	Tissue	0.02	1	mg/kg	75-125	70-130	30
6010C/PSEP	Thallium	7440-28-0	Tissue	0.3	10	mg/kg	75-125	70-130	30
6010C/PSEP	Tin	7440-31-5	Tissue	0.3	5	mg/kg	75-125	70-130	30
6010C/PSEP	Titanium	7440-32-6	Tissue	0.09	1	mg/kg	75-125	70-130	30
6010C/PSEP	Vanadium	7440-62-2	Tissue	0.07	1	mg/kg	75-125	70-130	30
6010C/PSEP	Zinc	7440-66-6	Tissue	0.06	1	mg/kg	75-125	70-130	30

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TABLE 3
Standard A for IRIS ICP-OES

Analyte	Source	Source Concentration (ppm)	Aliquot (mL)	Final Volume (mL)	Final Concentration (ppm)
Antimony	(1)	100	10	1000	1.0
Antimony	Elemental Stock	1000	4	1000	4.0
Arsenic	(1)	100	10	1000	1.0
Arsenic	Elemental Stock	1000	4	1000	4.0
Boron	(1)	100	10	1000	1.0
Cadmium	(1)	100	10	1000	1.0
Calcium	(1)	100	10	1000	1.0
Calcium	Elemental Stock	10000	0.4	1000	4.0
Chromium	(1)	100	10	1000	1.0
Cobalt	(1)	100	10	1000	1.0
Copper	(1)	100	10	1000	1.0
Iron	(1)	100	10	1000	1.0
Lead	(1)	100	10	1000	1.0
Lead	Elemental Stock	1000	4	1000	4.0
Magnesium	(1)	100	10	1000	1.0
Magnesium	Elemental Stock	1000	3	1000	3.0
Manganese	(1)	100	10	1000	1.0
Manganese	Elemental Stock	1000	1	1000	1.0
Molybdenum	(1)	100	10	1000	1.0
Molybdenum	Elemental Stock	1000	1	1000	1.0
Nickel	(1)	100	10	1000	1.0
Selenium	(1)	100	10	1000	1.0
Selenium	Elemental Stock	1000	4	1000	4.0
Silver	(1)	100	10	1000	1.0
Tin	Elemental Stock	1000	5	1000	5.0
Thallium	(1)	100	10	1000	1.0
Thallium	Elemental Stock	1000	9	1000	9.0
Titanium	(1)	100	10	1000	1.0
Vanadium	(1)	100	10	1000	1.0
Zinc	(1)	100	10	1000	1.0
Hydrochloric Acid	-	-	50	1000	5%
Nitric Acid	-	-	10	1000	1%

(1) Mixed Standard, QCS-26

TABLE 3 continued
Standard B for IRIS ICP-OES

Analyte	Source	Source Concentration (ppm)	Aliquot (mL)	Final Volume (mL)	Final Concentration (ppm)
Aluminum	Elemental Stock	10000	1	1000	10
Barium	Elemental Stock	10000	0.5	1000	5.0
Beryllium	Elemental Stock	1000	0.1	1000	0.1
Calcium	Elemental Stock	10000	5	1000	50
Iron	Elemental Stock	10000	5	1000	50
Lithium	Elemental Stock	1000	1	1000	1.0
Magnesium	Elemental Stock	10000	5	1000	50
Manganese	Elemental Stock	1000	10	1000	10
Phosphorus	Elemental Stock	10000	2	1000	20
Potassium	Elemental Stock	10000	2	1000	20
Silicon	Elemental Stock	10000	0.5	1000	5
Sodium	Elemental Stock	10000	2	1000	20
Strontium	Elemental Stock	1000	1	1000	1.0
HCl	-	-	50	1000	5%
HNO3	-	-	10	1000	1%

TABLE 4
Standard A for ICAP 6500 ICP-OES

Analyte	Source	Source Concentration (ppm)	Aliquot (mL)	Final Volume (mL)	Final Concentration (ppm)
Antimony	(1)	100	5	1000	0.5
Beryllium	(1)	100	5	1000	0.5
Boron	(1)	100	5	1000	0.5
Cadmium	(1)	100	5	1000	0.5
Calcium	(1)	100	5	1000	0.5
Chromium	(1)	100	5	1000	0.5
Cobalt	(1)	100	5	1000	0.5
Copper	(1)	100	5	1000	0.5
Iron	(1)	100	5	1000	0.5
Lead	(1)	100	5	1000	0.5
Magnesium	(1)	100	5	1000	0.5
Manganese	(1)	100	5	1000	0.5
Molybdenum	(1)	100	5	1000	0.5
Nickel	(1)	100	5	1000	0.5
Selenium	(1)	100	5	1000	0.5
Silver	(1)	100	5	1000	0.5
Tin	Elemental Stock	1000	0.5	1000	0.5
Thallium	(1)	100	5	1000	0.5
Titanium	(1)	100	5	1000	0.5
Vanadium	(1)	100	5	1000	0.5
Zinc	(1)	100	5	1000	0.5
Hydrochloric Acid	-	-	50	1000	5%
Nitric Acid	-	-	10	1000	1%

(1) Mixed Standard, QCS-26

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TABLE 4 continued
Standard B for ICAP 6500 ICP-OES

Analyte	Source	Source Concentration (ppm)	Aliquot (mL)	Final Volume (mL)	Final Concentration (ppm)
Aluminum	Elemental Stock	10000	2	1000	20
Arsenic	Elemental Stock	1000	2	1000	2
Barium	Elemental Stock	10000	2	1000	20
Calcium	Elemental Stock	10000	2	1000	20
Iron	Elemental Stock	10000	2	1000	20
Lithium	Elemental Stock	1000	2	1000	2
Magnesium	Elemental Stock	10000	2	1000	20
Phosphorus	Elemental Stock	10000	2	1000	20
Potassium	Elemental Stock	10000	2	1000	20
Silicon	Elemental Stock	10000	2	1000	20
Sodium	Elemental Stock	10000	2	1000	20
Strontium	Elemental Stock	1000	2	1000	2
HCl	-	-	50	1000	5%
HNO3	-	-	10	1000	1%

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TABLE 5
ICP ICV Standards

ICV1 Solution

Analyte	Source	Source Concentration (ppm)	Aliquot (mL)	Final Volume (mL)	Final Concentration (ppm)
Aluminum	QCP-CICV-1	1000	2.5	500	5.0
Antimony	QCP-CICV-2	500	2.5	500	2.5
Arsenic	QCP-CICV-3	500	2.5	500	2.5
Barium	QCP-CICV-1	1000	2.5	500	5.0
Beryllium	QCP-CICV-1	25	2.5	500	0.125
Cadmium	QCP-CICV-3	250	2.5	500	1.25
Calcium	QCP-CICV-1	2500	2.5	500	12.5
Chromium	QCP-CICV-1	100	2.5	500	0.5
Cobalt	QCP-CICV-1	250	2.5	500	1.25
Copper	QCP-CICV-1	125	2.5	500	0.625
Iron	QCP-CICV-1	500	2.5	500	2.5
Lead	QCP-CICV-3	500	2.5	500	2.5
Magnesium	QCP-CICV-1	2500	2.5	500	12.5
Manganese	QCP-CICV-1	250	2.5	500	1.25
Molybdenum	Elemental Stock	1000	1.0	500	2.0
Nickel	QCP-CICV-1	250	2.5	500	1.25
Potassium	QCP-CICV-1	2500	2.5	500	12.5
Selenium	QCP-CICV-3	500	2.5	500	2.5
Silver	QCP-CICV-1	125	2.5	500	0.625
Sodium	QCP-CICV-1	2500	2.5	500	12.5
Thallium	QCP-CICV-3	500	2.5	500	2.5
Titanium	Elemental Stock	1000	1.0	500	2.0
Vanadium	QCP-CICV-1	250	2.5	500	1.25
Zinc	QCP-CICV-1	250	2.5	500	1.25
Hydrochloric Acid	-	-	25	500	5%
Nitric Acid	-	-	5	500	1%

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TABLE 5 continued

ICVB Solution

Analyte	Source	Source Concentration (ppm)	Aliquot (mL)	Final Volume (mL)	Final Concentration (ppm)
Aluminum	Elemental Stock	1000	0.5	500	1
Boron	Elemental Stock	1000	2.5	500	5
Calcium	Elemental Stock	1000	2.5	500	5
Iron	Elemental Stock	1000	5	500	10
Lithium	Elemental Stock	1000	1	500	2
Magnesium	Elemental Stock	1000	2.5	500	5
Manganese	Elemental Stock	1000	5	500	10
Phosphorus	Elemental Stock	1000	2.5	500	5
Silicon	Elemental Stock	1000	2.5	500	5
Strontium	Elemental Stock	1000	1	500	2
Tin	Elemental Stock	1000	2.5	500	5
Hydrochloric Acid	-		25	500	5%
Nitric Acid	-		5	500	1%

TABLE 6
ICP Interference Check Solutions

ICSA Solution

Analyte	Source	Source Concentration (ppm)	Aliquot (mL)	Final Volume (mL)	Final Concentration (ppm)
Aluminum	CLPP-ICS-A	5000	50	500	500
Calcium	CLPP-ICS-A	5000	50	500	500
Iron	CLPP-ICS-A	2000	50	500	200
Magnesium	CLPP-ICS-A	5000	50	500	500
Hydrochloric Acid	-	-	25	500	5%
Nitric Acid	-	-	5	500	1%

ICSAB Solution

Analyte	Source	Source Concentration (ppm)	Aliquot (mL)	Final Volume (mL)	Final Concentration (ppm)
Aluminum	CLPP-ICS-A	5000	50	500	500
Antimony	Elemental Stock	1000	0.5	500	1
Barium	CLPP-ICS-B	50	5	500	0.5
Beryllium	CLPP-ICS-B	50	5	500	0.5
Cadmium	CLPP-ICS-B	100	5	500	1
Calcium	CLPP-ICS-A	5000	50	500	500
Chromium	CLPP-ICS-B	50	5	500	0.5
Cobalt	CLPP-ICS-B	50	5	500	0.5
Copper	CLPP-ICS-B	50	5	500	0.5
Iron	CLPP-ICS-A	2000	50	500	200
Lead	CLPP-ICS-B	100	5	500	1
Magnesium	CLPP-ICS-A	5000	50	500	500
Manganese	CLPP-ICS-B	50	5	500	0.5
Nickel	CLPP-ICS-B	100	5	500	1
Silver	CLPP-ICS-B	100	5	500	1
Vanadium	CLPP-ICS-B	50	5	500	0.5
Zinc	CLPP-ICS-B	100	5	500	1
HCl	-	-	25	500	0.05
HNO3	-	-	5	500	0.01

TABLE 7
IRIS Analytical Wavelengths

Analyte	Wavelength	
Aluminum	237.3	
Antimony	206.8	
Arsenic	189.0	
Barium	233.5	
Beryllium	313.0	
Boron	249.7	
Cadmium	226.5	
Calcium	317.9	
Calcium	211.2	High Line
Chromium	267.7	
Cobalt	228.6	
Copper	324.7	
Iron	259.9	
Iron	271.4	High Line
Lead	220.3	
Lithium	670.7	
Magnesium	279.5	
Magnesium	202.5	High Line
Manganese	257.6	
Manganese	293.9	High Line
Molybdenum	202.0	
Nickel	231.6	
Phosphorus	214.9	
Potassium	766.4	
Selenium	196.0	
Silicon	251.6	
Silver	328.0	
Sodium	589.5	
Strontium	407.7	
Thallium	190.8	
Tin	189.9	
Titanium	323.4	
Vanadium	310.2	
Zinc	206.2	

TABLE 8
ICAP 6500 Analytical Wavelengths

Analyte	Wavelength	
Aluminum	167.0	Low Line
Aluminum	394.4	
Antimony	206.8	
Antimony	217.5	Alternate
Arsenic	189.0	
Barium	455.4	
Beryllium	234.8	
Boron	249.6	
Cadmium	226.5	
Cadmium	214.4	Alternate
Calcium	315.8	
Calcium	393.3	Low Line
Chromium	267.7	
Cobalt	230.7	
Cobalt	228.6	Alternate
Copper	327.3	
Copper	224.7	Alternate
Iron	259.9	
Lead	220.3	
Lithium	670.7	
Magnesium	279.0	High Line
Magnesium	279.5	Low Line
Magnesium	285.2	
Manganese	257.6	
Manganese	260.5	High Line
Molybdenum	202.0	
Nickel	221.6	
Nickel	231.6	Alternate
Phosphorus	214.9	
Phosphorus	178.2	Alternate
Potassium	766.4	
Selenium	196.0	
Silicon	251.6	
Silver	328.0	
Sodium	588.9	Alternate
Sodium	589.5	

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TABLE 8
ICAP 6500 Analytical Wavelengths
continued

Strontium	407.7	
Thallium	190.8	
Tin	189.9	
Titanium	336.1	
Vanadium	292.4	
Zinc	206.2	
Zinc	213.8	Alternate

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STANDARD OPERATING PROCEDURE

for

DETERMINATION OF METALS AND TRACE ELEMENTS BY INDUCTIVELY COUPLED-MASS SPECTROMETRY (ICP-MS) – METHOD 200.8

SOP No.: MET-ICPMS

Revision: 14

Effective Date: March 16, 2012

Approved by: _____ Supervisor — 2/13/12 Date

_____ QA Manager — 2/13/12 Date

_____ Laboratory Director — 2/17/12 Date

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DETERMINATION OF METALS AND TRACE ELEMENTS BY INDUCTIVELY COUPLED-MASS SPECTROMETRY (ICP-MS) - METHOD 200.8

1. SCOPE AND APPLICATION

1.1. This procedure describes the steps taken for the analysis of soil, sludge, sediment, surface waters and drinking water using EPA Method 200.8 for a variety of elements. This SOP is intended to be used in conjunction with the EPA method as a guide to ICP-MS analysis. The complexity of the technique generally requires outside study of appropriate literature as well as specialized training by a qualified spectroscopist. The scope of this document does not allow for the in-depth descriptions of the relevant spectroscopic principles required for gaining a complete level of competence in this scientific discipline.

1.2. The Method Reporting Limits (MRLs) for common elements are listed in Table 1. The reported MRL may be adjusted if required for specific project requirements; however, the capability of achieving other reported MRLs must be demonstrated. Method Detection Limits (MDLs) which have been achieved are listed in Table 1. These may change as annual studies are performed.

2. METHOD SUMMARY

2.1. Prior to analysis, samples must be digested using appropriate sample preparation methods. The digestate is analyzed for the elements of interest using ICP spectrometry.

2.2. Method 200.8 describes the multi-elemental determination of analytes by ICP-MS. The method measures ions produced by a radio-frequency inductively coupled plasma. Analyte species originating in a liquid are nebulized and the resulting aerosol transported by argon gas into the plasma torch. The ions produced are entrained in the plasma gas and introduced, by means of an interface, into a mass spectrometer. The ions produced in the plasma are sorted according to their mass-to-charge ratios and quantified with a channel electron multiplier. Interferences must be assessed and valid corrections applied or the data flagged to indicate problems. Interference correction must include compensation for background ions contributed by the plasma gas, reagents, and constituents of the sample matrix.

2.3. Deviations from the reference method(s): This SOP contains no deviations from the reference methods.

3. DEFINITIONS

3.1. **Analysis Sequence** - Samples are analyzed in a set referred to as an analysis sequence. The sequence begins with instrument calibration followed by sample digestates interspersed with calibration standards.

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3.2. **Independent Calibration Verification (ICV)** - ICV solutions are made from a stock solution which is different from the stock used to prepare calibration standards and is used to verify the validity of the standardization.

3.3. **Matrix Spike** (MS) - In the matrix spike analysis, predetermined quantities of standard solutions of certain analytes are added to a sample matrix prior to sample digestion and analysis. The purpose of the matrix spike is to evaluate the effects of the sample matrix on the methods used for the analyses. Percent recoveries are calculated for each of the analytes detected.

3.4. **Matrix Spike Duplicate** (MSD) - In the matrix spike duplicate analysis, predetermined quantities of standard solutions of certain analytes are added to a sample matrix prior to sample digestion and analysis. The purpose of the matrix spike duplicate is to evaluate the effects of the sample matrix on the methods used for the analyses. Percent recoveries are calculated for each of the analytes detected. The relative percent difference between the matrix spikes is calculated and used to assess analytical precision.

3.5. **Duplicate Sample** (DUP) - A laboratory duplicate. The duplicate sample is a separate field sample aliquot that is processed in an identical manner as the sample proper. The relative percent difference between the samples is calculated and used to assess analytical precision.

3.6. **Method Blank** - The method blank is an artificial sample designed to monitor introduction of artifacts into the process. The method blank is carried through the entire analytical procedure.

3.7. **Continuing Calibration Verification Standard (CCV)** - A standard analyzed at specified intervals and used to verify the ongoing validity of the instrument calibration.

3.8. **Instrument Blank (CCB)** - The instrument blank (also called continuing calibration blank) is a volume of blank reagent of composition identical to the digestates. The purpose of the CCB is to determine the levels of contamination associated with the instrumental analysis.

4. INTERFERENCES

4.1. Isobaric elemental interferences in ICP-MS are caused by isotopes of different elements forming atomic ions with the same nominal mass-to-charge ratio (m/z). A data system must be used to correct for these interferences. This involves determining the signal for another isotope of the interfering element and subtracting the appropriate signal from the analyte isotope signal. Attention should be given to circumstances where very high ion currents at adjacent masses may contribute to ion signals at the mass of interest. Matrices exhibiting a significant problem of this type may require resolution improvement, matrix separation, or analysis using another isotope.

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4.2. Isobaric molecular and doubly-charged ion interferences in ICP-MS are caused by ions consisting of more than one atom or charge, respectively. Most isobaric interferences that could affect ICP-MS determinations have been identified in the literature.

5. SAFETY

5.1. All appropriate safety precautions for handling solvents, reagents and samples must be taken when performing this procedure. This includes the use of personnel protective equipment, such as, safety glasses, lab coat and the correct gloves.

5.2. Chemicals, reagents and standards must be handled as described in the CAS safety policies, approved methods and in MSDSs where available. Refer to the CAS Environmental, Health and Safety Manual and the appropriate MSDS prior to beginning this method.

5.3. Hydrochloric and/or Nitric Acid are used in this method. These acids are extremely corrosive and care must be taken while handling them. A face shield should be used while pouring acids. And safety glasses should be worn while working with the solutions. Lab coat and gloves should always be worn while working with these solutions.

5.4. High Voltage - The RF generator supplies up to 2000 watts to maintain an ICP. The power is transferred through the load coil located in the torch box. Contact with the load coil while generator is in operation will likely result in death. When performing maintenance on the RF generator, appropriate grounding of all HV capacitors must be performed as per manufacturer.

5.5. UV Light - The plasma is an intense source of UV emission, and must not be viewed with the naked eye. Protective lenses are in place on the instrument. Glasses with special protective lenses are available when direct viewing of the plasma is necessary.

6. SAMPLE COLLECTION, CONTAINERS, PRESERVATION, AND STORAGE

6.1. Samples are prepared using methods described in CAS SOPs MET-DIG or MET-3050B. Samples are generally received in the ICP-MS laboratory as 1% Nitric Acid digestates. Samples are stored in the appropriate volumetric containers.

6.2. Each time water samples are preserved after arrival at the lab, hold the samples for a minimum of 24 hours and verify the pH<2 prior to digestion.

6.3. Digestates originating from soil samples with greater than 60% solids are diluted prior to instrumental analysis by a factor of 5. This allows the analysis to achieve maximum sensitivity which results in optimum Method Reporting Limits (MRL). Following analysis, digestates are stored until all results have been reviewed. Digestates are brought to 3< pH<12 and disposed of through the sewer system 2 weeks after the data is reviewed.

7. APPARATUS & EQUIPMENT

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Instrument:	Thermo Elemental VG PQ Excell, or Thermo Electron X-Series
Nebulizer:	Meinhart (VG Excell)
Spray Chamber:	VG Peltier-cooled
Cones:	Nickel Sampler (1.0 mm orifice)
	Nickel Skimmer (0.75 mm orifice)
Peristaltic Pump:	Petec Perimax 12

8. STANDARDS, REAGENTS, & CONSUMABLE MATERIALS

8.1. All standards are prepared from NIST traceable standards as per SOP ADM-DATANTRY - *Making Entries into Logbooks and onto Analytical Records.* The manufacturer's expiration dates are used to determine viability of standards.

8.2. Stock Standard Solutions: The manufacturer, lot number, and expiration date of each stock standard is recorded in a bound logbook located in the room 113 of the Metals Department. Additionally each stock standard is given a unique, identifying name. Stock standards are typically purchased at 1000 mg/L concentrations.

8.2.1. Intermediate Standard Solutions: Intermediate mixed stock solutions are made from the individual stock standards described above. The individual components of each mixed solution are recorded in a bound logbook located in the ICP-MS laboratory and mixed solution is given a unique, identifying name.

8.2.2. A 1000 ug/L intermediate mixed stock standard containing the 200.8 list of metals, less silver, is first prepared. Add 1.0 mL of each purchased, single element, 1000 mg/L stock standard to a 1000 mL Class A volumetric flask filled approximately three quarters full with reagent water and 10 mL of Ultrex nitric acid. After all the additions are made the flask is diluted to volume with reagent water. A separate 1000 ug/L silver intermediate stock is prepared in the same fashion and in an amber flask. The expiration date for the intermediate standards in the earliest expiration date of the individual component stock solutions.

8.2.3. A second source intermediate stock standard is prepared from three premixed solutions purchased from Inorganic Ventures; QCP-CICV-1, QCP-CICV-2, and QCP-CICV-3. A 100 mL Class A volumetric flask is partially filled with reagent water and 5.0 mL of Ultrex nitric acid. Add 2.0 mL of QCP-CICV-1, and 1.0 mL each of QCP-CICV-2 and QCP-CICV-3 to the flask and dilute to volume with reagent water. Because of lower sensitivity, selenium may be prepared at a higher concentration.

8.2.4. Working Standards: Working calibration standards are made fresh daily from the intermediate standard solutions. Each individual intermediate standard used in the working standard is recorded in a bound logbook located in the ICP-MS laboratory, and the working standard solution is given a unique, identifying name.

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The unique I.D. of the working standard is used on the raw data to link the data to the subsequent prepared standards and ultimately the original purchased stock standard.

8.2.5. A 25 ug/L working CCV standard is prepared as follows: A 100 mL Class A volumetric flask is partially filled with reagent water and 1.0 mL of Ultrex nitric acid. Add 2.5 mL of the mixed intermediate stock and 2.5 mL of the silver intermediate stock and dilute to volume with reagent water.

8.2.6. The working ICV solution is prepared by first partially filling a 100 mL Class A volumetric flask with reagent water and 1.0 mL of Ultrex nitric acid. Add 0.5 mL of the ICV intermediate stock and dilute to volume with reagent water. The expiration dates for the stock and working ICV solutions are the earliest date of the purchased mixed solutions. If needed, add internal standards after dilution, mix and store in a FEP bottle. The ICV/QCS should be analyzed as needed to meet data-quality needs and a fresh solution should be prepared quarterly or more frequently as needed.

8.2.7. Internal Standards Stock Solution – Prepare 10 µg/mL solutions by making appropriate dilution of stock standards with reagent water, and store in a FEP bottle. Use this solution for addition to blanks, calibration standards and samples, or dilute by an appropriate amount using 1% (v/v) nitric acid, if the internal standards are being added by peristaltic pump.

9. PREVENTIVE MAINTENANCE

9.1. All maintenance activities are recorded in a maintenance logbook kept for each instrument. Pertinent information (serial numbers, instrument I.D., etc.) must be in the logbook. This includes the routine maintenance described in section 9. The entry in the log must include: date of event, the initials of who performed the work, and a reference to analytical control.

9.2. CAS/Kelso maintains a service contract with the instrument manufacturer that allows for an unlimited number of service calls and full reimbursement of all parts and labor.

9.3. Most routine maintenance and troubleshooting is performed by CAS staff. Preventive maintenance activities listed below should be performed when needed as determined by instrument performance (i.e. stability, sensitivity, etc.) or by visual inspection. Other maintenance or repairs may, or may not require factory service, depending on the nature of the task.

- cone removal and cleaning
- removal and cleaning of ICP glassware and fittings
- checking and cleaning RF contact strips
- checking air filters and cleaning if necessary
- checking the oil mist filters and cleaning if necessary

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- checking the rotary pump oil and adding or changing if necessary
- removal and cleaning of extraction lens
- removal and cleaning of ion lens stack
- replace the electron multiplier as necessary

10. RESPONSIBILITIES

10.1. It is the responsibility of the analyst to perform the analysis according to this SOP and to complete all documentation required for data review. Analysis and interpretation of the results are performed by personnel in the laboratory who have demonstrated the ability to generate acceptable results utilizing this SOP. This demonstration is in accordance with the training program of the laboratory. Final review and sign-off of the data is performed by the department supervisor/manager or designee.

10.2. It is the responsibility of the department supervisor/manager to document analyst training. Documenting method proficiency, as described in the applicable EPA method, is also the responsibility of the department supervisor/manager.

11. PROCEDURE

11.1. The following parameters are monitored to assure awareness of changes in the instrumentation that serve as signals that optimum performance is not being achieved, or as indicators of the physical condition of certain consumable components (i.e. EMT and cones).

11.1.1. Multiplier High Voltage

The PQ Excell and X-Series instruments use a dual mode detector (pulse count/analog). These detectors are adjusted by the instrument software.

11.2. Optimization

11.2.1. Gas Flows

11.2.1.1. Allow a period of not less than 30 minutes for the instrument to warm-up.

11.2.1.2. Aspirate a mixed element tune solution into the plasma and monitor the instrument output signal at mass In 115 on the ratemeter. Adjust the nebulizer and auxiliary flows to obtain maximum signal. Adjust the tension screw on the peristaltic pump to obtain minimum noise in the analytical signal. Record flow rates and note any large variances.

11.2.1.3. Note: Significant differences in flow rates will be observed for different torches and cones.

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11.2.2. Ion Lens Setting

While monitoring the output signal of a tune solution at mass In 115 on the ratemeter, adjust the ion lenses to obtain maximum sensitivity. Refer to the instrument manual for details on performing the adjustments.

11.2.3. Mass Calibration

Aspirate a solution of Be, Mg, Co, In, Pb, and U using the Mass Calibration program in the VG software with these elements identified in the program as the points used for mass calibrating. Refer to the instrument manual for details pertaining to the mass calibration procedure.

11.2.4. Resolution Check

Using the spectra created during the mass calibration procedure, check the resolution. The resolution must be less than 1 AMU at 5% of peak height, but should be set at approximately 0.75 AMU at 5% of the peak height. The PQ Excell instrument checks the resolution at 10% peak height which is more rigorous than at 5%.

11.2.5. Stability Check

Using the mixed element solution form the mass calibrations check perform a short-term stability check. Instrument stability must be demonstrated by running the tuning solution a minimum of five times with resulting relative standard deviations of absolute signals for all analytes of less than 5%.

11.3. Internal Standards

11.3.1. Internal standardization is used in all analyses to correct for instrument drift and physical interferences. Internal standards are added to all controls and samples prior to analysis. This may be achieved by directly adding an aliquot of the internal standards, or by mixing with the solution prior to nebulization using a peristaltic pump. Internal standards must be present in all samples, standards and blanks at identical levels and in a like manner.

11.3.2. The internal standards used will vary depending on the sample matrix, known interferences, and other factors. For full mass range scans a minimum of three internal standards must be used. The concentration of the internal standard is set such that good precision is obtained in the measurement of the isotope used for data correction and to minimize the possibility of correction errors if the internal standard is naturally present in the sample. The typical internal standards are listed below.

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Internal Standard	Mass	Possible Limitation
^{6}Lithium	6	a
Scandium	45	polyatomic ion interference
Yttrium	89	a,b
Rhodium	103	
Indium	115	isobaric interference by Sn
Terbium	159	
Holmium	165	
Lutetium	175	
Bismuth	209	a

a - May be present in environmental samples.
b - In some instruments Yttrium may form measurable amounts of YO^{+} (105 amu) and YOH^{+} (106 amu). If this is the case, care should be taken in the use of the cadmium elemental correction equation.

11.3.3. Calibration using internal standards and internal standard ratios is performed by the instrument software using calculations shown in Attachment A.

11.4. Analytical Run

11.4.1. Select the correct method.

11.4.2. Nebulize Standard 0 (Blank) into the plasma. Allow 1-2 minutes for system to equilibrate prior to establishing baseline.

11.4.3. Follow directions on computer screen to perform standardization. Operator will sign and date the first page of standardization.

11.4.4. Perform the analysis in the order listed below, with no more than 10 samples between CCV/CCBs.

Initial Calibration Verification (ICV)
Continuing Calibration Verification (CCV)
Initial Calibration Blank (ICB)
Continuing Calibration Blank (CCB)
CRA – Method Reporting Limit (or project specified) level*
Analyze ten samples
CCV
CCB
Analyze ten samples
CCV
CCB
Repeat sequence as required to complete analytical run

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* Note: For AFCEE projects, the CRA standard concentrations will be equal to the project MRLs.

12. QA/QC REQUIREMENTS

12.1. Initial Precision and Recovery Validation

12.1.1. The accuracy and precision of the procedure must be validated before analyses of samples begin, or whenever significant changes to the procedures have been made. To do this, four water samples are spiked with the LCS spike solution, then prepared and analyzed.

12.1.2. The average percent recovery must be 85-115% (for water, and within LCS limits for soils) and the RSD< 30%.

12.1.3. Initial Demonstration of Performance must be performed by each analyst performing sample analysis and documented in laboratory records.

12.2. Linear calibration ranges – Establish the upper limit of the linear calibration range for each analyte by measuring the signal responses from a minimum of three different concentration standards, one of which is close to the upper limit of the linear range. Care should be taken to avoid potential damage to the detector when performing these tests. The linear calibration range which may be used for sample analysis should be judged by the analyst from the resulting data. The upper linear range limit should be an observed signal no more than 10% below the level extrapolated from lower standards. Analyte concentrations that are greater than 90% of the determined upper linear range limit must be diluted and reanalyzed. The linear ranges should be verified whenever a change in instrument hardware or operating conditions results in a change in analytical performance of the instrument.

12.3. Independent Calibration Verification Standard (EPA 200.8 QCS) - When beginning the use of this procedure, on a quarterly basis or as required to meet data-quality needs, verify the calibration standards and acceptable instrument performance with the preparation and analyses of a ICV/QCS (Section 8.2.6). To verify the calibration standards the determined mean concentration from three analyses of the ICV/QCS must be within ±10% of the stated ICV/QCS value. If the ICV/QCS is used for determining acceptable on-going instrument performance, analysis of the ICV/QCS prepared to a concentration of 100 µg/L must be within ±10% of the stated value or within the acceptance limits listed in Table 8 of Method 200.8, whichever is the greater. If not, an immediate second analysis of the ICV/QCS is recommended to confirm unacceptable performance. If the calibration standards and/or acceptable instrument performance cannot be verified, the source of the problem must be identified and corrected before either proceeding on with the initial determination of method detection limits or continuing with on-going analyses.

12.4. Method Detection Limits and Method Reporting Limits

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12.4.1. A method detection limit (MDL) study must be undertaken before analysis of samples can begin. To establish detection limits that are precise and accurate, the analyst must perform the following procedure. Spike seven blank matrix (water or soil) samples with MDL spiking solution at a level below the MRL. Follow the analysis procedures in Section 11 to analyze the samples.

12.4.2. Calculate the average concentration, and the standard deviation of the concentrations for each analyte. Calculate the MDL for each analyte. Refer to the CAS *SOP Performing Method Detection Limit Studies and Establishing Limits of Detection and Quantification (ADM-MDL).* The MDL study must be verified annually.

12.4.3. Limits of Quantification (LOQ)

12.4.3.1. The laboratory establishes a LOQ for each analyte as the lowest reliable laboratory reporting concentration or in most cases the lowest point in the calibration curve which is less than or equal to the desired regulatory action levels, based on the stated project requirements. Analysis of a standard or extract prepared at the lowest point calibration standard provides confirmation of the established sensitivity of the method. The LOQ recoveries should be within 70-130% of the true values to verify the data reporting limit. Refer to the CAS SOP *Performing Method Detection Limit Studies and Establishing Limits of Detection and Quantification (ADM-MDL)*

12.4.4. The Method Reporting Limits (MRLs) used at CAS are the routinely reported lower limits of quantitation which take into account day-to-day fluctuations in instrument sensitivity as well as other factors. These MRLs are the levels to which CAS routinely reports results in order to minimize false positive or false negative results. The MRL is normally two to ten times the method detection limit.

12.5. Ongoing QC Samples required are described in the CAS-Kelso Quality Assurance Manual and in the SOP for Sample Batches. Additional QC Samples may be required in project specific quality assurance plans (QAPP). General QC Samples are:

12.5.1. A Continuing Calibration Verification (CCV) and Continuing Calibration Blank (CCB) are analyzed after every 10 samples. The control limit for CCV recoveries is ±10%. If the control limits are exceeded, the instrument will be recalibrated and the previous 10 samples reanalyzed.

12.5.2. As per method 200.8, a digested duplicate and matrix spike are analyzed at a frequency of one per 10 samples (or one per batch if fewer than 10 samples). The matrix spike recovery and relative percent difference will be calculated while

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analysis is in progress. The control limit for matrix spikes is 70-130% as specified by method 200.8, duplicate RPD limits are 20% for waters and 30% for soils. Client specific QC criteria may supercede the limits listed in EPA Method 200.8 or the CAS QA Manual. If the control limits are exceeded, the samples will be redigested and reanalyzed, unless matrix interference or non-homogeneity can be established as the cause. In these instances, the data and report will be noted accordingly.

12.5.3. Laboratory Control Samples are analyzed at a frequency of 5% or one per batch, whichever is greater. The control limits for aqueous LCS recoveries is 85-115%, soil control limits are specific to the reference material being used. Client specific QC criteria may supercede these limits. If the control limits are exceeded, the samples will be redigested and reanalyzed.

12.5.4. A Laboratory Fortified Blank (LFB) spiked at the level of the MRL is digested and analyzed with every drinking water batch of samples. The advisory criterion of 50 – 150 % is used.

12.6. Internal standards responses are monitored throughout the analysis sequence. Ratios of the internal standards responses against each other should also be monitored routinely. This information is used to identify potential problems caused by mass dependent drift, errors incurred in adding the internal standards or increases in the concentrations of individual internal standards caused by background contributions from the sample. The absolute response of any one internal standard must not deviate more than 60-125% of the original response in the calibration blank. If deviations greater than these are observed, flush the instrument with the rinse blank and monitor the responses in the calibration blank. If the responses of the internal standards are now within the limit, take a fresh aliquot of the sample, dilute by a further factor of two, add the internal standards and reanalyze. If after flushing the response of the internal standards in the calibration blank exceed the internal standard acceptance limits, terminate the analysis and determine the cause of the drift. Possible causes of drift may be a partially blocked sampling cone or a change in the tuning condition of the instrument.

12.7. Instrument Detection Limits (IDL) and linear ranges are performed quarterly, Method Detection Limits (MDLs) are performed annually. These will be calculated and made available to the ICP-MS operator.

12.8. Common isobaric interferences are corrected by the instrument software using the equations listed in Attachment B. Monitoring of multiple isotopes for a single element provides a mechanism for identifying isobaric interferences. The scope of this document does not allow for an in-depth discussion/training course pertaining to the interpretation of ICP-MS spectra. The final review and approval of all data is performed by qualified spectroscopists. Refer to the Interferences section of EPA Method 200.8 for additional descriptions of possible interferences and the mechanisms required for adequately compensating for their effects.

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12.9. Note that the nomenclature of certain QC samples in the method differs from that of CAS, but the function of those samples is equivalent in both cases.

13. DATA REDUCTION, REPORTING, AND REVIEW

13.1. Using the results of integrations for applicable masses, the instrument software calculates the measured (instrument) sample concentration using an internal standard calibration algorithm (Attachment A). This calculation includes appropriate interference corrections.

13.1.1. Target analytes are corrected by internal standardization via three possible scenarios. The analysis record (raw data) will indicate the IS masses and target element masses used for quantitation.

- If the mass of the target analyte is less than the mass of the first internal standard (the IS of lowest mass) the target analyte references this internal standard alone using the algorithm described in Attachment A.

- If the mass of the target analyte is between the mass of two internal standards the target analyte references both internal standards using linear interpolation as described in Attachment A.

- If the mass of the target analyte is greater than the mass of the last internal standard (the IS of highest mass) the target analyte references this internal standard alone using the algorithm described in Attachment A.

Note: For Arizona samples, all analyses must use the technique of internal standard normalization by referencing a single internal standard (rather than interpolation).

13.1.2. Typical internal standard references when using internal standard normalization by referencing a single internal standard are listed below:

Analyte	Internal Standard Reference	Analyte	Internal Standard Reference
Aluminum	Li-6	Manganese	Ga-71
Antimony	In-115	Molybdenum	In-115
Arsenic	Ga-71	Nickel	Ga-71
Barium	Lu-175	Selenium	Ga-71
Beryllium	Li-6	Silver	In-115
Cadmium	In-115	Thallium	Lu-175
Chromium	Ga-71	Uranium	Bi-209
Cobalt	Ga-71	Vanadium	Ga-71
Copper	Ga-71	Zinc	Ga-71
Lead	Lu-175		

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13.2. Using the chosen standardization technique, the data system will calculate solution concentrations using the procedures and calculations described in the attached *PlasmaLab Calculations* document (Attachment A).

13.3. Calculate sample results for each analyte using the data system printouts (showing instrument concentrations) and digestion information. The digestion and dilution information is entered into the data system. The data system then uses the calculations below to generate a sample result.

Aqueous samples are reported in µg/L:

$$\mu g/L\,(Sample) = C_d \text{ x Digestion DF x Post Digestion Dilution Factor}$$

where: C_d = Concentration measured at the instrument in ug/L (in digestate).
DF = Dilution factor

Solid samples are reported in mg/Kg:

$$mg/Kg\ (Sample) = C_d \text{ x Post Digestion DF } x \frac{Digestion\ Vol.(ml)}{Sample\ wt.(g)} x \frac{1mg}{1000ug} x \frac{1L}{1000ml} x \frac{1000g}{1Kg}$$

where: C_d = Concentration measured at the instrument in ug/L (in digestate).
DF = Dilution factor

13.4. Data Review and Reporting

13.4.1. Production

The ICP-MS operator performs an initial review of the data, followed by a peer review. The report is then generated. A senior spectroscopist performs a final review of the data and associated report. The data is then placed in the holding file until all analyses are complete. When the work order is complete, a final review is performed and the data is delivered to the project management department.

Tier III or higher type deliverables require a diskette of the ICP-MS data to be generated. The file is also stored on the hard drive of the ICP-MS until the data package has been generated.

13.4.2. Non-production

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Method Development/Research and Development studies are performed under the direction of a senior spectroscopist. All associated data is scrutinized by the senior spectroscopist. Original raw data and associated records are archived in the analytical project file.

14. CONTINGENCIES FOR HANDLING OUT-OF-CONTROL OR UNACCEPTABLE DATA

Corrective action measures applicable to specific analysis steps are discussed in the applicable section of this (and other applicable) SOP(s). Also, refer to the SOP for *Corrective Action* for correct procedures for identifying and documenting such data. Procedures for applying data qualifiers are described in the SOP for *Report Generation* or in project-specific requirements.

15. METHOD PERFORMANCE

This method was validated through single laboratory studies of accuracy and precision. Refer to the reference method for additional available method performance data.

The method detection limit (MDL) is established using the procedure described in the SOP for *The Determination of Method Detection Limits and Limits of Detection* (ADM-MDL). Method Reporting Limits are established for this method based on MDL studies and as specified in the CAS Quality Assurance Manual.

16. POLLUTION PREVENTION

It is the laboratory's practice to minimize the amount of solvents and reagents used to perform this method wherever technically sound, feasibly possible and within method requirements. Standards are prepared in volumes consistent with the laboratory use in order to minimize the volume of expired standards to be disposed of. The threat to the environment from solvents and/or reagents used in this method may be minimized when recycled or disposed of properly.

17. WASTE MANAGEMENT

17.1. The laboratory will comply with all Federal, State and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions as specified in the CAS *EH&S Manual*.

17.2. This method uses acid. Waste acid is hazardous to the sewer system and to the environment. All acid waste must be neutralized to a pH of 5-9 prior to disposal down the drain. The neutralization step is considered hazardous waste treatment and must be documented on the treatment by generator record. See the CAS *EH&S Manual* for details.

18. TRAINING

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18.1. Refer to the SOP ADM-TRANDOC *Documentation of Training* for standard procedures.

18.2. A minimum of two senior level spectroscopists are to be maintained on staff at all times. Senior spectroscopists are defined as individuals with a minimum of ten years combined education and experience in, or related to atomic spectroscopy. Of those ten years, a minimum of two years of ICP-MS experience is required.

18.3. To maintain expertise in current technology, senior staff members are encouraged to attend technical seminars containing significant information relevant to ICP-MS as they are available. In addition, senior spectroscopists are also encouraged to attend training secessions offered periodically by the ICP-MS instrument manufacturers.

18.4. On-the-job-training occurs daily with the senior spectroscopists providing direction to new operators. The physical operation of the equipment is relatively simple. The data reduction and troubleshooting requires extensive experience that can only be gained by hands-on operation of the instrument and assisted evaluation of raw data.

18.5. Training outline

18.5.1. Review literature (see references section). Read and understand the SOP. Also review the applicable MSDS for all reagents and standards used. Following these reviews, observe the procedure as performed by an experienced analyst at least three times.

18.5.2. The next training step is to assist in the procedure under the guidance of an experienced analyst. During this period, the analyst is expected to transition from a role of assisting, to performing the procedure with minimal oversight from an experienced analyst.

18.5.3. Perform initial precision and recovery (IPR) study as described above for water or soil samples. Summaries of the IPR are reviewed and signed by the supervisor. Copies may be forwarded to the employee's training file. For applicable tests, IPR studies should be performed in order to be equivalent to NELAC's Initial Demonstration of Capability.

18.5.4. Training and proficiency is documented in accordance with the SOP ADM-TRANDOC.

19. METHOD MODIFICATIONS

19.1. There are no known modifications in this laboratory standard operating procedure from the reference method.

20. REFERENCES

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20.1. Thermo Elemental Instrument Manuals

20.2. USEPA, Methods for Determination of Metals in Environmental Samples, Method 200.8, Revision 5.4, May 1994.

21. CHANGES SINCE THE LAST REVISION

21.1. Sec 7: Omitted VG Plasma Quad S; updated spray chamber to VG peltier cooled and omitted TJA Fixed Cross Flow, Gilson minipuls 3 – to reflect current use.
21.2. Sec 8 – reformatted
21.3. Sec 9.1 - updated
21.4. Sec 11.1.1 - Removed HT2 and HT1 paragraphs
21.5. Sec 11.2.5- Sentence in parenthesis
21.6. Sec 11.3.2 Concentration column was omitted in the section due to the use of instrument specific concentrations.
21.7. Sec 11.4.4 - Updated to “analyze ten samples.
21.8. Sec 12.1.1 updated
21.9. Sec 12.4 - updated
21.10. Sec 12.5.4 -added LFB prepared for Drinking Water Analysis.
21.11. Sec 19 is new
21.12. Table I updated

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CAS/KELSO DATA QUALITY OBJECTIVES – TABLE 1

METHOD	ANALYTE	CAS No.	MATRIX	MDLa	MRL	LODb	LOQc	UNITS	(LCS %Rec.)	(MS %Rec)	(% RPD)
200.8	Aluminum	7429-90-5	Water	0.3	2	1	2	ug/L	85-115	70-130	20
200.8	Antimony	7440-36-0	Water	0.02	0.05	0.05	0.05	ug/L	85-115	70-130	20
200.8	Arsenic	7440-38-2	Water	0.1	0.5	0.25	0.5	ug/L	85-115	70-130	20
200.8	Barium	7440-39-3	Water	0.02	0.05	0.05	0.05	ug/L	85-115	70-130	20
200.8	Beryllium	7440-41-7	Water	0.006	0.02	0.01	0.02	ug/L	85-115	70-130	20
200.8	Bismuth	7440-69-9	Water	0.006	0.1	0.013	0.1	ug/L	85-115	70-130	20
200.8	Boron	7440-42-8	Water	0.2	0.5	0.25	0.5	ug/L	85-115	70-130	20
200.8	Cadmium	7440-43-9	Water	0.005	0.02	0.01	0.02	ug/L	85-115	70-130	20
200.8	Chromium	7440-47-3	Water	0.04	0.2	0.1	0.2	ug/L	85-115	70-130	20
200.8	Cobalt	7440-48-4	Water	0.006	0.02	0.01	0.02	ug/L	85-115	70-130	20
200.8	Copper	7440-50-8	Water	0.02	0.1	0.1	0.1	ug/L	85-115	70-130	20
200.8	Lead	7439-92-1	Water	0.005	0.02	0.02	0.02	ug/L	85-115	70-130	20
200.8	Manganese	7439-96-5	Water	0.006	0.05	0.05	0.05	ug/L	85-115	70-130	20
200.8	Molybdenum	7439-98-7	Water	0.008	0.05	0.03	0.05	ug/L	85-115	70-130	20
200.8	Nickel	7440-02-0	Water	0.03	0.2	0.1	0.2	ug/L	85-115	70-130	20
200.8	Selenium	7782-49-2	Water	0.3	1	0.5	1	ug/L	85-115	70-130	20
200.8	Silver	7440-22-4	Water	0.004	0.02	0.01	0.02	ug/L	85-115	70-130	20
200.8	Thallium	7440-28-0	Water	0.005	0.02	0.01	0.02	ug/L	85-115	70-130	20
200.8	Tin	7440-31-5	Water	0.02	0.1	0.025	0.1	ug/L	85-115	70-130	20
200.8	Uranium	7440-61-1	Water	0.007	0.02	0.01	0.02	ug/L	85-115	70-130	20
200.8	Vanadium	7440-62-2	Water	0.03	0.2	0.1	0.2	ug/L	85-115	70-130	20
200.8	Zinc	7440-66-6	Water	0.2	0.5	0.5	0.5	ug/L	85-115	70-130	20

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CAS/KELSO DATA QUALITY OBJECTIVES – TABLE 1 (cont.)

METHOD	ANALYTE	CAS No.	MATRIX	MDLa	MRL	LODb	LOQc	UNITS	(LCS %Rec.)	(MS % Rec.)	(% RPD)
200.8	Aluminum	7429-90-5	Soil	0.4	2	0.5	2	mg/kg	41-158	70-130	30
200.8	Antimony	7440-36-0	Soil	0.02	0.05	0.025	0.05	mg/kg	50-150	70-130	30
200.8	Arsenic	7440-38-2	Soil	0.06	0.5	0.13	0.5	mg/kg	78-122	70-130	30
200.8	Barium	7440-39-3	Soil	0.005	0.05	0.006	0.05	mg/kg	81-119	70-130	30
200.8	Beryllium	7440-41-7	Soil	0.003	0.02	0.005	0.02	mg/kg	83-117	70-130	30
200.8	Cadmium	7440-43-9	Soil	0.004	0.02	0.005	0.02	mg/kg	81-119	70-130	30
200.8	Chromium	7440-47-3	Soil	0.03	0.2	0.1	0.2	mg/kg	80-119	70-130	30
200.8	Cobalt	7440-48-4	Soil	0.003	0.02	0.005	0.02	mg/kg	82-118	70-130	30
200.8	Copper	7440-50-8	Soil	0.08	0.1	0.05	0.1	mg/kg	83-116	70-130	30
200.8	Lead	7439-92-1	Soil	0.009	0.05	0.013	0.05	mg/kg	79-121	70-130	30
200.8	Manganese	7439-96-5	Soil	0.03	0.05	0.03	0.05	mg/kg	81-119	70-130	30
200.8	Molybdenum	7439-98-7	Soil	0.02	0.05	0.025	0.05	mg/kg	75-125	70-130	30
200.8	Nickel	7440-02-0	Soil	0.03	0.2	0.1	0.2	mg/kg	81-118	70-130	30
200.8	Selenium	7782-49-2	Soil	0.2	1	0.25	1	mg/kg	74-143	70-130	30
200.8	Silver	7440-22-4	Soil	0.008	0.02	0.01	0.02	mg/kg	81-129	70-130	30
200.8	Thallium	7440-28-0	Soil	0.003	0.02	0.005	0.04	mg/kg	79-120	70-130	30
200.8	Vanadium	7440-62-2	Soil	0.02	0.2	0.03	0.2	mg/kg	79-121	70-130	30
200.8	Zinc	7440-66-6	Soil	0.2	0.5	0.5	0.5	mg/kg	73-121	70-130	30

a Method Detection Limits are subject to change as new MDL studies are completed.

a MDL is the smallest analyte concentration that can be demonstrated to be different from zero with 99% confidence

b The LOD is the smallest amount of a substance that must be present in a sample in order to be detected with 99% confidence. Verification is acceptable if the response is > 3x instrument noise.

c The LOQ is the lowest concentration of a substance that produces a quantitative result within specified limits of precision and bias.

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Attachment A

Internal Standard Calibration Calculations

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1.3 Internal Standard Correction

All samples which have internal standards defined are internal standard (IS) corrected. Every acquired peak has an IS correction factor (ISCF) calculated (this may be 1.0 if there are no internal standards defined or IS correction is not required for the analyte). This ISCF value is then multiplied by the interference corrected ICPS value of the peak to produce the IS corrected ICPS value (ISICPS).

(i) $ISICPS = ICICPS * ISCF$

1.3.1 Internal standard analytes

For all analytes that are defined as internal standards, the ISCF is calculated as a ratio of sensitivities as follows

(ii) $ISCF = S_r / S_t$

where S_r and S_t denote the reference sensitivity and target analyte sensitivity respectively.

The sensitivity of the target analyte is given by

(iii) $S_t = ICICPS_t / C_d$

where C_d is the defined concentration.

The reference sensitivity is obtained from one of the following methods, in order of priority:

i) A reference internal standard in the reference sample.
The reference sample is the first sample in the preceding calibration block. If the corresponding analyte in the reference sample is defined as an internal standard and it has a valid mean ISICPS value then it is used to obtain the reference sensitivity which is given by

(iv) $S_r = \text{mean } ISICPS_r / C_{dr}$

Where C_{dr} is the defined concentration of the internal standard in the reference sample.

(Note – this means that, ideally, the set of internal standards in the reference sample should contain all the internal standards that are to be used in any subsequent samples.)

ii) A reference calibration.
If a reference is not found in (i) the reference calibration may be used. The reference calibration is the analyte calibration in the preceding calibration block. If the calibration is valid then the reference sensitivity is given by the slope of the calibration (C_1), (see section 1.4)

(v) $S_r = C_1$

(Note – for calibration standard samples that form the calibration block, the internal

standard corrected value is used to construct the calibration curve. The calibration curve cannot therefore be used as an internal standard reference point for these samples. If an internal standard within these samples has no corresponding internal standard in the reference sample (i) then the internal standard will just reference itself (iv).)

iii) A semi-quantitative response curve.
If a reference is not found in (i) or (ii) the reference semi-quantitative response curve may be used. The semi-quantitative response curve from the preceding calibration block may be used to obtain an estimate of an analyte's sensitivity (S_{sq}) (see section 1.5.3). This may then be used as the reference sensitivity (as noted in (ii) calibration standards may not be corrected using this method).

(vi) $S_r = S_{sq}$

iv) Self reference.
In situations where an internal standard is unable to obtain a reference in (i), (ii) or (iii), the reference is taken from the mean sensitivity of the analyte within the target sample i.e. the internal standard correction is within sample and cannot correct for any drift between samples. (Note – this will be the case for the first sample in a calibration block which will then be used as a reference for subsequent samples.)

(vii) $S_r = \text{mean ICICPS}_t / C_d$

1.3.2 Analytes which are not internal standards

Analytes which are not defined as internal standards have an ISCF calculated from the ISCF of the internal standard analytes within the same acquired run. The method of calculating the ISCF may be selected from the following:

i) Reference
For analytes with internal standard correction by reference, the ISCF is set to the same value as a valid selected internal standard analyte within the run.

(viii) $ISCF = ISCF_r$

ii) Interpolation
If the IS correction method is interpolation, the ISCF of the target analyte derived by interpolation from the bracketing internal standard analytes (by mass). Using the mass (M) of the target analyte, the ISCF is derived from

(viiii) $ISCF = ISCF_1 + (ISCF_2 - ISCF_1)((M - M_1) / (M_2 - M_1))$

where $ISCF_1$ and $ISCF_2$ are the respective correction factors of the bracketing internal standards at a lower mass (M_1) and higher mass (M_2).

(Note – analytes not bracketed by internal standards (e.g. at a lower mass than the first internal standard or a higher mass than the last internal standard) will have the ISCF set equal to the first or last internal standard respetively.)

iii) None

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If this option is chosen (or there are no valid internal standards in the run) no internal standard correction is performed.

(x) ISCF = 1.0

1.3.3 Internal standard correction of survey data

Survey scan data may be acquired in a different way to main scan data. This can lead to large differences in the measured sensitivity for an analyte between a main run and a survey run within a sample. To account for this and produce comparable data the survey data is corrected relative to the internal standard corrected main scan data. For all analytes defined as internal standards within the main scan, if a corresponding peak exists in the survey scan it is used as an internal standard and the ISCF value is calculated to scale the ICICPS value in the survey to the ISICPS value in the main data.

(xi) ISCF = mean $ISICPS_{main}$ / $ICICPS_{survey}$

The remaining survey peaks are then corrected by interpolation (see 1.3.2 part ii).

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STANDARD OPERATING PROCEDURE

for

SAMPLE PREPARATION OF BIOLOGICAL TISSUE FOR METALS ANALYSIS BY GFAA, ICP-OES, AND ICP-MS

SOP No.: MET-TDIG
Revision: 3

Effective date: February 13, 2012

Approved by: _____ Supervisor — 1/17/12 Date

_____ QA Manager — 1-17-12 Date

_____ Laboratory Director — 1/17/12 Date

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SAMPLE PREPARATION OF BIOLOGICAL TISSUE FOR METALS ANALYSIS BY GFAA, ICP-OES, AND ICP-MS

1. SCOPE AND APPLICATION

This procedure describes techniques used for sample preparation and acid digestion of biological tissue samples. This procedure is applicable to the analysis of biological tissue for heavy metals. The procedure provides a convenient and efficient digestion/dissolution technique which allows for the simultaneous or sequential analysis of the sample for metals. The digestates may be analyzed by graphite furnace atomic absorption (GFAA), Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES), or Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). The procedure includes, but is not restricted to, the metals listed in Table 1.

2. METHOD SUMMARY

A representative tissue sample is lyophilized, blended, then sub-sampled for conventional oven digestion. Oxidation is brought about by the use of concentrated nitric acid in a Teflon closed vessel. The digestate is then analyzed for metallic constituents by GFAA, ICP-OES, or ICP-MS methods

3. DEFINITIONS

3.1. **Batch** - A batch of samples is a group of environmental samples that are prepared and/or analyzed together as a unit with the same process and personnel using the same lot(s) of reagents. It is the basic unit for analytical quality control.

3.1.1. Preparation Batch - A preparation batch is composed of one to twenty field samples, all of the same matrix, with a maximum time between the start of processing of the first and last samples in the batch to be 24 hours.

3.2. **Method Blank (MB)** - The method blank or Preparation Blank (PB) is an artificial sample is designed to monitor the introduction of artifacts into the analytical process. The method blank is carried through the entire analytical procedure.

3.3. **Laboratory Control Samples (LCS)** – The LCS is laboratory created sample to which known amounts target analytes are added. The LCS is prepared and analyzed in exactly the same manner as the samples. The percent recovery is compared to established limits and assists in determining whether the batch is in control.

3.4. **Matrix Spike/Duplicate Matrix Spike (MS/DMS)** - In the matrix spike analysis, predetermined quantities of target analytes is added to a sample matrix prior to sample preparation and analysis. The purpose of the matrix spike is to evaluate the effects of the sample matrix on the method used for the analysis. Samples are split into duplicates, spiked, and analyzed as a MS/DMS pair. Percent recoveries are calculated for each of

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the analytes detected. The relative percent difference (RPD) between the duplicate spikes (or samples) is calculated and used to assess analytical precision. The concentration of the spike should be at levels specified by a project analysis plan.

3.5. **Laboratory Duplicates (DUP)** – Duplicates are additional replicates of samples that are subjected to the same preparation and analytical scheme as the original sample. The relative percent difference (RPD) between the sample and its duplicate is calculated and used to assess analytical precision.

3.6. **Standard Reference Material (SRM)** – A material with specific certification criteria and is issued with a certificate or certificate of analysis that reports the results of its characterizations and provides information regarding the appropriate use(s) of the material. An SRM is prepared and used for three main purposes: (1) to help develop accurate methods of analysis; (2) to calibrate measurement systems used to facilitate exchange of goods, institute quality control, determine performance characteristics, or measure a property at the state-of-the-art limit; and (3) to ensure the long-term adequacy and integrity of measurement quality assurance programs.

4. INTERFERENCES

Refer to the determinative method for a discussion of interferences.

5. SAFETY

5.1. Follow all CAS safety practices as described in the CAS Safety Manual.

5.2. Each chemical compound or reagent should be treated as a potential health hazard. Exposure to these chemicals should be reduced to the lowest possible level. A reference file of material safety data sheets is available to all personnel involved in these analyses. CAS also maintains a file of OSHA regulations regarding the safe handling of the chemicals specified in this method.

5.3. Nitric Acid is extremely corrosive. Care should be taken while working with this chemical. Personal protective equipment shall include safety glasses (with side shields), gloves and a lab coat. Follow normal precautions as per the CAS Safety Manual.

6. SAMPLE, COLLECTION, PRESERVATION AND STORAGE

Samples are typically collected in plastic containers and iced or refrigerated at $4 \pm 2°C$ from time of collection until preparation or analysis. Sample may be frozen at $\leq$-10ºC or as specified by project requirements.

7. APPARATUS AND EQUIPMENT

7.1. Lyophilizing apparatus, LABCONCO Model 7948040 freeze dryer.

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7.2. Conventional laboratory oven capable of precise temperature control at 105^{o}C.

7.3. Analytical balance capable of weighing to 1 mg.

7.4. 50 mL graduated poly tubes.

8. STANDARDS AND REAGENTS

8.1. Reagent grade chemicals shall be used in all tests. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lowering the accuracy of the determination. The preparation for all laboratory prepared reagents and solutions must be documented in a laboratory logbook. Refer to the SOP *Reagent/Standards Login and Tracking (ADM-RTL)* for the complete procedure and documentation requirements.

8.1.1. Reagent water - ASTM Type I or Type II water

8.1.2. Concentrated nitric acid.

8.2. Standards

8.2.1. Stock standard solutions may be purchased from a number of vendors. All reference standards, where possible, must be traceable to SI units or NIST certified reference materials. The vendor-assigned expiration date is used.

8.2.2. Metals spiking solutions: Five solutions are needed to prepare the matrix spiking standards: SS1, SS2, SS3, SS4, and SS5.

8.2.3. Follow the formulations laid out in Table 2. These solutions are prepared in acid rinsed Class A volumetric flasks using purchased custom mixed standards or 1000 ppm single analyte standards. Aliquots are made using acid rinsed Class A volumetric pipettes of the appropriate size.

8.2.3.1. SS1 (Al, Ag, Ba, Be, Cd, Co, Cr, Cu, Fe, Pb, Mn, Ni, Sb, V, and Zn): Fill a 1000 mL volumetric flask approximately half full with reagent water, add 50 mL of nitric acid and mix. Next add 100 mL of the custom mixed standard (CAS-CAL-14) purchased from “Inorganic Ventures”. In addition add 50 mL of 1000 ppm Antimony. Dilute to volume with reagent water, mix thoroughly and transfer to a 1000 mL Teflon bottle for storage. The solution expiration date is determined by the earliest expiration date of any single component in the solution.

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8.2.3.2. SS2 (As, Cd, Pb, Se, Tl and Cu): Fill a 500 mL volumetric flask approximately half full with reagent water, add 25 mL of nitric acid and mix. Next add 2.0 mL each of 1000 ppm Arsenic, Cadmium, Lead, Selenium, Thallium and Copper. Dilute to volume with reagent water, mix thoroughly and transfer to a 500 mL Teflon bottle for storage. The solution expiration date is determined by the earliest expiration date of any single component in the solution.

8.2.3.3. SS3 (As, Se, and Tl): Fill a 500 mL volumetric flask approximately half full with reagent water, add 25 mL of nitric acid and mix. Next add 50 mL each of 1000 ppm Arsenic, Selenium, and Thallium. Dilute to volume with reagent water, mix thoroughly and transfer to a 500 mL Teflon bottle for storage. The solution expiration date is determined by the earliest expiration date of any single component in the solution.

8.2.3.4. SS4 (B, Mo): Fill a 500 mL volumetric flask approximately half full with reagent water, add 25 mL of nitric acid and mix. Next add 50 mL each of 1000 ppm Boron and Molybdenum. Dilute to volume with reagent water, mix thoroughly and transfer to a 500 mL Teflon bottle for storage. The solution's expiration date is determined by the earliest expiration date of any single component in the solution.

8.2.3.5. SS5 (K, Na, Mg, Ca): Fill a 1000 mL volumetric flask approximately half full with reagent water, add 10 mL of nitric acid and mix. Next add 100 mL of the custom mixed standard (CAS-CAL-14) purchased from "Inorganic Ventures". In addition add 20 mL of 10000 ppm Phosphorus, Sodium, Magnesium, Calcium. Dilute to volume with reagent water, mix thoroughly and transfer to a 1000 mL Teflon bottle for storage. The solution expiration date is determined by the earliest expiration date of any single component in the solution.

9. PREVENTIVE MAINTENANCE

9.1. All maintenance activities are recorded in a maintenance logbook kept for the freeze drying instrument. Pertinent information (serial numbers, instrument I.D., etc.) must be in the logbook. Maintenance entries should include date, symptom of problem, corrective actions, and description of maintenance, date, and name. The log should contain a reference to return to analytical control.

9.2. Centrifuge tubes must be thoroughly pre-cleaned with 1:4 HCl, and rinsed with DI water. All laboratory equipment used for trace metals analysis shall be stored in the clean room, and shall not be used for any other purpose.

9.3. Routine cleaning of the sample handling and digestion apparatus is necessary. Refer to the SOP for Metals Laboratory Glassware Cleaning.

10. RESPONSIBILITIES

10.1. It is the responsibility of the analyst to perform the analysis according to this SOP and to complete all documentation required for data review. Analysis and interpretation of the results are performed by personnel in the laboratory who have demonstrated the ability to generate acceptable results utilizing this SOP. This demonstration is in accordance with the training program of the laboratory. Final review and sign-off of the data is performed by the department supervisor/manager or designee.

10.2. It is the responsibility of the department supervisor/manager to document analyst training. Documenting method proficiency, as described in the SOP *Documentation of Training* (ADM-TRANDOC), is also the responsibility of the department supervisor/manager.

10.3. When new or unfamiliar situations develop, it is also the responsibility of the employee to immediately notify the section supervisor, project manager, or the laboratory director for quick resolution of all issues. Due to the nature of the work, it is important that all work be documented, department supervisors, project managers and when necessary, the client, be contacted immediately when questions arise.

11. PROCEDURE

11.1. Obtain a representative tissue sample that will yield ~ 300 mg of freeze-dried solids.

Note: This is designed to be a general guideline. Approximately 300 mg of dry sample is typically required to obtain the desired detection limits that often are necessary for tissue analysis.

11.2. Slice the sample into thin pieces prior to filling the drying vessel. Homogenize the sample before freeze drying.

11.3. Cap the vessel and freeze the sample in a conventional freezer. Note: Using the Labconco Model 7948040 freeze dryer, the samples do not need to be frozen prior to freeze drying.

11.4. When the sample is frozen, remove from freezer and follow the manufacturer's instructions for operation of the freezer dryer.

11.5. Blend the dry solids to obtain a homogeneous sample. The sample may be stored dry until digestion.

11.6. Sample Digestion

11.6.1. Transfer 300 mg of dried sample, weighed to the nearest 0.1 mg, to a 50 mL decomposition vessel.

11.6.2. Add 4.5mL concentrated nitric acid to the vessel.

11.6.3. Follow the manufacturer's instructions for the use of the decomposition vessels. Place the vessel in a conventional oven at 105^{o}C for a minimum of 12 hours. If using the conventional oven option, monitor oven temperatures for each batch and record this data onto the appropriate benchsheet.

11.6.4. Cool the vessel, open to relieve pressure and vent the gases. If Antimony, Silver or Tin are target analytes, then add 10 ml of deionized water to the cooled vessel. Re-cap and tighten the vessel. Return to the oven for an additional hour.

11.6.5. Cool the vessel, open to relieve pressure and vent the gasses. Transfer the sample to a volumetric container and dilute to 30 ml. The sample is ready for analysis.

12. QA/QC REQUIREMENTS

12.1. Ongoing QC Samples required are described in the CAS-Kelso Quality Assurance Manual and in the SOP for Sample Batches. Additional QC Samples may be required in project specific quality assurance plans (QAPP). General QC Samples are:

12.1.1. Method Blank

12.1.1.1. A method blank is prepared in an empty digestion vessel and digested with every batch of 20 (or fewer) samples to demonstrate that there are no method interferences. If the method blank shows any hits above the reporting limit, corrective action must be taken. Corrective action includes recalculation, reanalysis, system cleaning, or re-extraction and reanalysis. For some project specific needs, exceptions may be noted and method blank results above the MRL may be reported for common lab contaminants.

12.1.2. Lab Control Sample (LCS)

12.1.2.1. The laboratory control sample is composed of both a SRM and a laboratory created sample. The laboratory created LCS is created by spiking the elements defined in the project plan into a digestion vessel. The LCS is designed to monitor the accuracy of the procedure.

12.1.2.2. Prepare one laboratory control samples (LCS) and a duplicate laboratory control sample (DLCS) with every batch of 20 (or fewer) samples whichever is more frequent.

12.1.2.3. Analyze a standard reference material (SRM) at 5% frequency or one per batch, whichever is more frequent. Standard Reference Material (SRM's) should be representative of the tissue sample being analyzed.

12.1.3. Sample duplicates

12.1.3.1. Samples analyzed by methods 6010, 6020 or GFAA require one sample duplicate at with every batch of 20 (or fewer) samples. Methods 200.7 and 200.8 require one sample duplicate at with every batch of 10 (or fewer) samples.

12.1.4. Matrix Spike

12.1.4.1. A matrix spike (MS) is prepared and analyzed with every batch of 20 (or fewer) samples if analyzed by methods 6010, 6020 or GFAA. Methods 200.7 and 200.8 require one sample duplicate at with every batch of 10 (or fewer) samples.

12.1.4.2. The MS is prepared by adding a known volume of the matrix spike solution to the sample and determining the spiked sample concentration. Spikes should be added directly to the dry sample. Spike solutions should be multi-element with analyte concentrations high enough to minimize volume added to sample.

13. REPORTING

13.1. Refer to the SOP for *Data Reporting and Report Generation* for reporting guidelines.

14. DATA REVIEW AND ASSESSMENT

14.1. Digestion data sheets, including weights and volumes used are completed and a batch lot number is assigned and attached to the data sheet. The Manufacturer's lot numbers for the reagents used are added to the digestion data sheet. (See appendix A)

14.2. Spiking sheets are completed including all spike data and volumes of spiking solutions used.

14.3. Following primary data interpretation and calculations, all data is reviewed by a secondary analyst. Following generation of the report, the report is also reviewed. Refer to the *SOP for Laboratory Data Review Process* for details. The person responsible for final review of the data report and/or data package should assess the overall validity and quality of

the results and provide any appropriate comments and information to the Project Chemist to inclusion in the report narrative.

15. CORRECTIVE ACTION

15.1. Refer to the SOP for *Corrective Action.* Personnel at all levels and positions in the laboratory are to be alert to identifying problems and nonconformities when errors, deficiencies, or out-of-control situations are detected.

15.1.1. Handling out-of-control or unacceptable data

On-the-spot corrective actions that are routinely made by analysts and result in acceptable analyses should be documented as normal operating procedures; specific documentation need be made other than notations in laboratory maintenance logbooks, runlogs, for example.

15.1.2. Documentation of nonconformity must be done using a Corrective Action Report (NCAR) when: a) corrective action is not taken or not possible b) corrective action fails to correct an out-of-control problem.

16. POLLUTION PREVENTION AND WASTE MANAGEMENT

16.1. It is the laboratory's practice to minimize the amount of solvents, acids, and reagents used to perform this method wherever feasibly possible. Standards are prepared in volumes consistent with methodology and only the amount needed for routine laboratory use is kept on site. The threat to the environment from solvents and/or reagents used in this method can be minimized when recycled or disposed of properly.

16.2. The laboratory will comply with all Federal, State, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions as specified in the CAS Environmental Health and Safety Manual.

17. TRAINING

17.1. Training outline (See the SOP for *Documentation of Training*)

17.1.1. Read and understand the SOP/Method. Also review the applicable MSDS for all reagents and standards used. Following these reviews, observe the procedure as performed by an experienced analyst at least three times.

17.1.2. The next training step is to assist in the procedure under the guidance of an experienced analyst. During this period, the trainee is expected to transition from a role of assisting, to performing the procedure with minimal oversight from an experienced analyst. This incremental step in training shall be documented at

each step by use of the Training Plan or a modified version of the Training Plan outlined in the SOP for *Documentation of Training.*

17.1.3. Perform an initial precision and recovery (IPR) study for tissue samples. Summaries of the IPR are reviewed and signed by the Technical Director. Along with the completed Training Plan, copies are forwarded to the employee's training file. For applicable tests, IPR studies should be performed in order to be equivalent to NELAC's Initial Demonstration of Capability.

17.2. Training is documented following the SOP *for Documentation of Training*.

NOTE: When the analyst training is documented by the supervisor on internal training documentation forms, the supervisor is acknowledging that the analyst has read and understands this SOP and that adequate training has been given to the analyst to competently perform the analysis independently.

18. REFERENCES

Recommended Guidelines for Measuring Metals in Puget Sound Marine Water, Sediment, and Tissue Samples; April 1997.

19. CHANGES SINCE THE LAST REVISION

19.1. Sec 3.1 is new
19.2. Sec 3.6 is new
19.3. Sec 8.1 is new
19.4. Sec 8.2 – updated standards and added detail to procedure for spiking standards prep
19.5. Sec 9.2 – is new
19.6. Sec 12.1.2 and 12.1.4– added QC for 200.7 & 200.8
19.7. Sec 12.1 – removed reference to DoD QSM and added detail to entire section

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Table 1

Selected Elements and Analysis Procedures for Tissue Samples

Element	GFAA	ICP-OES	ICP-MS
Aluminum		√	√
Antimony		√	√
Arsenic	√		√
Barium		√	√
Beryllium		√	√
Boron		√	
Cadmium	√	√	√
Calcium		√	
Chromium		√	√
Cobalt		√	√
Copper		√	√
Iron		√	
Lead	√	√	√
Lithium		√	
Magnesium		√	
Manganese		√	√
Molybdenum		√	√
Nickel		√	√
Potassium		√	
Phosphorus		√	
Selenium	√		
Silver		√	√
Sodium		√	
Strontium		√	
Thallium	√	√	√
Tin		√	√
Vanadium		√	
Zinc		√	√

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Table 2

METALS SPIKING SOLUTIONS CONCENTRATIONS FORM

Solution Name	Element	mLs of 1000ppm Solution	Final Volume	Solution Conc. mg/L	Concentration in the digest mg/L
SS1	HNO3	50.0	1000ml	-	
	Al	100*	1000ml	200	2
	Ag	100*	1000ml	5	0.05
	Ba	100*	1000ml	200	2
	Be	100*	1000ml	5	0.05
	Cd	100*	1000ml	5	0.05
	Co	100*	1000ml	50	0.5
	Cr	100*	1000ml	20	0.2
	Cu	100*	1000ml	25	0.25
	Fe	100*	1000ml	100	1
	Pb	100*	1000ml	50	0.5
	Mn	100*	1000ml	50	0.5
	Ni	100*	1000ml	50	0.5
	Sb	50	1000ml	50	0.5
	V	100*	1000ml	50	0.5
	Zn	100*	1000ml	50	0.5
SS2	HNO3	25.0	500ml	-	
GFAA SPIKE	As	2.0	500ml	4	0.04
	Cd	2.0	500ml	4	0.04
	Pb	2.0	500ml	4	0.04
	Se	2.0	500ml	4	0.04
	Tl	2.0	500ml	4	0.04
	Cu	2.0	500ml	4	0.04
SS3	HNO3	25.0	500ml	-	
	As	50.0	500ml	100	1
	Se	50.0	500ml	100	1
	Tl	50.0	500ml	100	1
SS4	HNO3	25	500ml	-	
	B	50	500ml	100	1
	Mo	50	500ml	100	1
SS5	HNO3	10.0	200ml	-	
	K**	20	200ml	1000	10
	Na**	20	200ml	1000	10
	Mg**	20	200ml	1000	10
	Ca**	20	200ml	1000	10

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Operation of the Freeze Drier Labconco 7948040

Operation Checklist

The following checklist should be followed after each use of the Stoppering Tray Dryer.

1. Wipe out the interior of the Stoppering Tray Dryer chamber with a paper towel to remove any moisture or debris.
2. Wipe the interior of the collector chamber (base unit)of the freeze dry system with a paper towel to remove any accumulated moisture.
3. Check the collector chamber drain hose on the freeze dry system (base unit) to ensure that the hose is free of moisture and that the drain plug is securely installed.
4. Using a paper towel, wipe the freeze dry system collector chamber (base unit) lid gasket and the Stoppering Tray Dryer door gasket to remove any dirt and contaminants that could cause a vacuum leak. Vacuum grease is not required on the door gasket or collector lid gasket to obtain proper vacuum seal.

Starting the Freeze Dry cycle

1. On the left side of the Stoppering Tray Dryer unit flip the black switch to the on position. On the left side of the base unit flip the black switch to the on position. On the base unit that the condensing coil is located push the manual button. The collector temperature must reach -40°C before the vacuum pump can be turned on.

2. Place the containers holding the pre-frozen samples onto the trays in the Stoppering Tray Dryer unit on top of the base unit. When closing the door pick up left hand bottom corner of door while closing. Turn the door handle all the way to the right. The door should be covering the entire black gasket attached to the Stoppering tray unit. Make sure that the vacuum release valve on Stoppering Tray Dryer unit is turned to the closed position. Press the mode (see attached diagram) button until the green light is on for Automatic on the Stoppering Tray Dryer unit. Press the run stop button on the Stoppering Tray Dryer unit P1 will appear in the display and the amber led button by the run stop button will light. Once the Stoppering Tray Dryer unit reaches -40°C it will hold at that temperature for one hour and forty eight minutes. After one hour and forty eight minutes the vacuum pressure in the display should decrease. If the vacuum is not decreasing press the vacuum on the collector unit button and open and close the door lifting up the bottom left hand corner of the door while closing. Turn the door handle all the way to the right. Press the vacuum button to restart the vacuum.

3. When the vacuum pressure (mBar) is the same on the Stoppering tray dryer and the base unit the samples have finished the freeze drying process (typically after 48 hours, depending on mass of tissue). Press the vacuum button on the collector unit to turn the vacuum pump off. Flip the switch on the side of the base unit to off. Flip the switch on the side of the Stoppering Tray Dryer unit to off. Open the chamber by moving the vacuum release control on the front of the

Stoppering Tray Dryer unit to the open position. When the sound of air through the back fill port is no longer audible, the chamber door is ready to open.

Alarms

A number of unusual events may occur during a lyophilization procedure that can adversely effect the operation of the Stoppering Tray Dryer. If an event occurs, the alarm indicator will flash and the beeper will sound. The beeper will automatically mute itself after one minute. The specific alarm can be identified observing the display. The following “out of specification” conditions will initiate an alarm:

System Temperature Variations
Once the system temperature has stabilized for 20 minutes, if the manual set point temperature or automatic hold temperature varies more than ±2°C as measured by the system temperature sensor, the Red Alarm indicator and word “TMP” on the display will flash until the end of the run.

Vacuum
Once the system vacuum is low and stabilize at a point where it changes less that 0.020mBar in 5 minutes if the vacuum changes more than 0.500mBar , the red Alarm indicator and the word “VAC” on the display will flash until the end of the run.

System Temperature Set Point
If during a ramp mode the system temperatures stabilize without reaching the set point temperature, the control will enter the next Hold mode. The Red Alarm indicator will flash and the program indicator “Px” on the display will flash until the end of the run.

Power Failure
If a power failure occurs while a run in is progress, the Red Alarm indicator and Run/Stop indicator will flash when the power in restored. Once the power is restored, the process will continue as programmed until completion. Pressing Run/Stop cancels the flashing warning.

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Vacuum Pump

The oil in the Vacuum pump should be checked before every use. It must be changed if it is cloudy, shows particles or is discolored. The useful life of the vacuum pump oil can be extended if the vacuum pump is operated for an extended period of time after a freeze dry run.

Changing the Vacuum pump oil:

Pump oil is located in the ICPMS Instrument Laboratory.

1. Make sure the Vacuum pump is turned off
2. Remove the front cover of the bottom unit of the freeze drying unit by pressing up on the metal clasp located on the bottom of the front cover in the middle. Use a small screwdriver.
3. Slide the Pump out far enough to allow a 1 L plastic container to placed under the drain on the front of the Vacuum pump.
4. Remove the Grey cap on top of the Vacuum pump.
5. Remove the Grey plug on the bottom of the Vacuum pump.
6. After all of the used oil has drained out of the pump, pour a small amount of the unused oil into the vacuum pump with the drain plug removed to rinse out the oil reservoir. Place the used oil in the **Used Oil** container located in the ICPMS Instrumentation Laboratory.
7. Place the drain plug on the vacuum pump (hand tighten) fill the pump up with approx 400 mls of oil the visib should be 1 inch from the top mark on the outside of the vacuum pump. Screw the grey cap back onto the top vacuum pump (hand tighten).

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STANDARD OPERATING PROCEDURE

for

TISSUE SAMPLE PREPARATION

SOP Code: MET-TISP
Revision: 8

Effective Date: February 20, 2012

Approved By:

Supervisor		1/23/12 Date
QA Manager		1/23/12 Date
Laboratory Director		1/23/12 Date

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DOCUMENT CONTROL	
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Initials:	Date:

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Standard Operating Procedure for Tissue Sample Preparation

1. PURPOSE

This standard operating procedure describes procedures for the initial preparation of tissue samples prior to sample analysis. Customer-specific contracts or statement of works (SOWs) with alternate procedures will take precedence over this SOP.

2. APPLICABILITY

This SOP is intended to provide guidance for the preliminary preparation of tissue samples prior to the sample aliquoting and analytical preparation described in individual analytical SOPs. The procedures described in this SOP also apply to compositing and subsampling of tissue samples for analyses to be subcontracted.

3. DEFINITIONS

3.1. Sample: The material presented to the laboratory for analysis or testing.

3.2. Sample Aliquot: A representative part or portion of a sample for analysis which is a fraction of the whole sample. See subsampling also.

3.3. Compositing: The process by which sample aliquots from two or more samples are united to form a combined sample which is subsequently analyzed.

3.4. Subsampling: The process by which a representative portion is obtained from a whole sample.

3.5. Service Request: The service request (SR) is a document prepared at the time of sample receipt and summarizes sample analysis and reporting instructions about a customer's sample(s).

3.6. QAPP: Quality Assurance Project Plan document provided by the client specifically written for their project.

3.7. VOC Analyses: Volatile organic compounds (VOC) analyses, including halogenated and aromatic volatile organic compounds and gasoline range organics (GRO) analyses.

3.8. Non-VOC Analyses: Any analysis other than a VOC analysis.

4. DISCUSSION

4.1. Tissue samples are inherently heterogeneous requiring special considerations in order to obtain a truly representative sample aliquot for analysis. This SOP provides guidance for handling tissue samples prior to the sample preparation steps described in analytical SOPs. This SOP applies to samples delivered to the lab in whole body form or in the form of pre-dissected tissues.

4.2. The sample handling strategy must consider:

- what analyses are to be performed (metals, organics, or both, and VOC or non-VOC),
- how much sample is available
- are the analyses to be performed on individual samples or composite homogenates,
- are the analyses to be performed on whole body, edible portions or specific organs, and
- are any of the analyses going to be subcontracted which may require subsampling.

4.3. Proper preparation and handling of tissue samples is required to obtain a representative sample, avoid contamination, and to ensure loss of sample and target constituents is minimized.

5. SAFETY

5.1. All appropriate safety precautions for handling solvents, reagents and samples must be taken when performing this procedure. This includes the use of personnel protective equipment, such as, safety glasses, lab coat and the correct gloves.

5.2. Chemicals, reagents and standards must be handled as described in the CAS safety policies, approved methods and in MSDSs where available. Refer to the CAS Environmental, Health and Safety Manual and the appropriate MSDS prior to beginning this method.

6. SAMPLE COLLECTION, CONTAINERS, PRESERVATION, AND STORAGE

Refer to the determinative method.

7. REAGENTS AND STANDARDS

7.1. Not applicable to this procedure

8. APPARATUS AND EQUIPMENT

Note: Refer to the Procedure section for specific equipment used based on the determinative analysis to be performed. The use of implements and surfaces may vary depending on the analyses to be performed.

8.1. Hobart Food Chopper, or comparable device.

8.2. Tissumizer.

8.3. Waring blender, or similar device.

8.4. Freeze-drier, Labonco or equivalent.

8.5. Glass or PTFE cutting boards.

8.6. Knives and cutting implements – refer to Procedure section.

8.7. Standard laboratory glassware (beakers, scintillation vials, etc.)

8.8. VOA vial – pre-cleaned, 40ml with Teflon-lined cap.

8.9. Pre-cleaned glass jars with PTFE lined lids, various sizes.

8.10. Gloves: Should be talc free and of non-contaminating materials.

8.11. Heavy duty aluminum foil.

9. RESPONSIBILITIES

9.1. It is the responsibility of the analyst to perform the analysis according to this SOP and to complete all documentation required for data review. Analysis and interpretation of the results are performed by personnel in the laboratory who have demonstrated the ability to generate acceptable results utilizing this SOP. This demonstration is in accordance with the training program of the laboratory. Final review and sign-off of the data is performed by the department supervisor/manager or designee.

9.2. Sample custodians, together with project chemists and department supervisors, are responsible for documenting any required sample preparation (including the percent solids or percent lipids determination if required) on the service request. All personnel preparing tissue samples should be familiar with the contents of this document prior to commencing work.

9.3. Tissue sample preparation is to be performed only by lab analysts instructed in the proper handling techniques outlined in this SOP. It is the responsibility of the analyst to perform this procedure to complete all documentation required for data review.

10. PREVENTIVE MAINTENANCE

10.1. No specific maintenance steps are needed other than normal cleaning and inspection of apparatus.

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10.2. For organics samples, polypropylene and polyethylene (plastic) surfaces, implements, and containers are a potential source of adsorption and contamination and should not be used. Gloves should be talc free and of non-contaminating materials.

11. PROCEDURE

11.1. Sample Login

Any special sample handling must be noted on the service request and on a label attached to the sample itself. During sample receiving, a sample custodian must follow the procedures described in Section 11.1.1.

11.1.1. Tissue Samples with Limited Quantity

An assessment of the required sample quantity should be made by the project chemist when taking delivery of the sample. This assessment must take into consideration the tests, the required detection limits and the necessary quality assurance samples. If the quantity of sample given to the laboratory is insufficient for the analyses requested, the sample custodian will, along with the project chemist estimate the total amount of sample available. A "LIMITED SAMPLE VOLUME" tag is attached to the sample on which is recorded the estimated sample quantity.

In some cases it may be beneficial to perform sample preparations as described in this SOP prior to estimating the sample amount. In this case, the analyst prepring the sample will provide the project chemist with an estimate of the amount available.

The project chemist must determine if limited sample quantity exists and set the priorities for the analyses and, if possible, estimate the quantity of sample to be *used for each test*. This information is to be documented and placed in the project file and on the service request to communicate to the laboratory staff. For example, 8081 use 10 g; metals use 1g.

NOTE. Samples that are quantity limited and require multiple analyses must be identified as soon as possible. Optimally, this should happen during sample login; however, discovery at any time should trigger appropriate actions as described in Section 11.1.1.

11.2. Sample Homogenization

This section outlines the steps for preparing homogenous samples of whole fish, edible fish (fillets), edible shellfish, worm composite homogenates, eggs, and plant tissues.

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11.2.1. Samples for Organics Analyses:

Equipment used for the processing of tissue samples for organics analyses should be of stainless steel, anodized aluminum, glass or polytetrafluorethylene (PTFE). Polypropylene and polyethylene (plastic) surfaces, implements, and containers are a potential source of adsorption and contamination and should not be used. Gloves should be talc free and of non-contaminating materials. Filleting should be done on glass or PTFE cutting boards that are cleaned properly between samples or on cutting boards that are covered with heavy duty aluminum foil (hexane rinsed) that is changed between samples. Tissue should be handled with precleaned, high quality, corrosion-resistant stainless steel instruments. Fillets or homogenate should be stored in cleaned glass jars of suitable dimensions with PTFE lined lids. If the sample is to be analyzed for VOCs, the homogenization steps should be performed on sample tissue that is partially frozen or chilled. An aliquot of the homogenate should be placed in a clean 40ml voa vial and labelled “FOR VOA ANALYSIS ONLY”.

Prior to handling each sample, utensils, cutting boards and containers should be washed in a detergent hot water solution and rinsed with tap water, hexane, and DI water. Precleaned, certified sample containers may be used without further cleaning. If the sample is to be analyzed for VOCs, methanol is substituted for the rinsing of implements with acetone and the hexane rinsing of the aluminum foil. Exposure to solvent vapors must be minimized.

11.2.2. Samples for Metals Analyses:

Equipment used in the processing of samples for metals analyses should be of PTFE, ceramic, polypropylene or polyethylene. Filleting should be performed on PTFE cutting boards which are cleaned after each sample. Knives with titanium or high quality stainless steel blades may be used for tissue resections. Tissue should be stored in glass jars with PTFE lined lids.

Prior to sample handling, utensils, cutting boards and containers should be washed in a detergent hot water solution, rinsed with tap water, 25% HCL (except metal utensils), and DI water. Precleaned, certified sample containers may be used without further cleaning.

11.2.3. Samples for both Metals and Organics Analyses:

If the sample is to be prepared for both organics and metals, care must be taken to use equipment and cleaning procedures that are non-contaminating for both. Quartz, ceramic, glass and PTFE are recommended materials for sample processing equipment. Knives with titanium or high quality stainless steel blades may be used for tissue resections. Glass or PTFE cutting boards should be used. If the sample is to be analyzed for VOC’s, the homogenization steps should be performed on sample tissue that is partially frozen or chilled. An aliquot of the homogenate

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should be placed in a clean 40ml voa vial and labeled "FOR VOA ANALYSIS ONLY".

Prior to handling each sample, utensils, cutting boards and containers should be washed in a detergent hot water solution and rinsed with tap water, acetone, methanol, or hexane (as appropriate), and DI water. Precleaned, certified sample containers may be used without further cleaning. Non-metallic surfaces and utensils should also be rinsed with 25% HCL followed by DI water. If the sample is to be analyzed for VOCs, methanol is substituted for the rinsing of implements with acetone. Exposure to solvent vapors must be minimized.

11.2.4. Sample Preparation

Each tissue sample may be homogenized in the original glass bottle container if there is sufficient space to allow thorough mixing. If homogenization is not achievable in the original container, place the entire sample contents into a clean glass jar. Generally, liquids contained in the container are to be considered part of the sample. If the sample requires size reduction prior to homogenization, chop the sample into the 1-2" chunks using a titanium or stainless steel bladed knife. Large samples may require the use of industrial food processors such as a Hobart Food Chopper, or comparable device. Size-reduced chunks of tissue are thoroughly homogenized to a paste-like consistency using a Tissumizer, Waring blender, or similar device until it reaches a paste-like consistency. Transfer the sample paste to a glass jar for storage and freeze until ready for sample extraction. The new sample container is labeled with the sample I.D., the word "homogenized", initialed, and dated.

11.2.4.1. Whole Fish Tissue

Samples may be frozen in the field or in the laboratory. While still partially frozen, rinse the fish with DI water to remove extraneous materials and liquids. Cut the fish into appropriate size chunks and mechanically macerate the sample using cutting tools appropriate for the size of the sample and the analysis type. If necessary, process fish tissue chunks through the Hobart Food Chopper. To ensure thorough mixing, divide the ground sample into quarters, mix opposite quarters and then mix halves. Homogenize sample using a Tissumizer or Waring blender until it reaches a paste-like consistency. Transfer the sample paste to a glass jar for storage and freeze until ready for sample extraction. The new sample container is labeled with the sample I.D., the word "homogenized", initialed, and dated.

11.2.4.2. Edible Fish Tissue

If the client or QAPP indicates that only edible tissue be analyzed, the fish must be filleted. If the sample arrives prefilleted, the sample tissue may be frozen before processing. If the sample is not yet filleted, the sample should

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remain chilled until the filleting is completed. Freezing can result in the contamination of edible tissues from the bursting of internal organs. Fish having ruptured internal organs should be noted on the prep benchsheet and the Project Chemist consulted. Rinse the fish with DI water to remove extraneous materials and liquids. Remove scales from scaled fish or skin from non-scaled fish. Rinse the fish again prior to filleting. A separate or clean cutting board should be used for filleting. Gloves should be changed between samples. Carefully remove the fillets from the carcass by following the steps outlined in Appendix A. Care should be taken to avoid contaminating fillet with inadvertent puncture of internal organs. Cut the fillet tissue into appropriate size chunks and mechanically macerate the sample using cutting and grinding tools appropriate for the size of the sample and the analysis type. Proper selection of maceration equipment must consider the potential contaminants, sample size/volume and amount of tissue likely to be lost in using the equipment.

Divide the ground sample into quarters, mix opposite quarters and then mix halves. Again homogenize the sample using an appropriate blending mixer. Continue repeating this process until the sample is truly homogenous and no chunks of tissue remain. Freeze sample until ready for extraction.

11.2.4.3. Shellfish Tissue

Shellfish should be frozen as soon as possible after receipt by the laboratory unless samples can be prepared within 48 hours of sampling. Edible portions of various shellfish are described below and resection described in Appendix B. Thawing of frozen shellfish samples should be kept to a minimum during tissue removal to avoid loss of liquids. Shellfish should be rinsed with DI water prior to tissue removal to dislodge external debris. When multiple organisms constitute a single sample, the edible tissues are collected, composited and homogenized.

11.2.4.3.1. Bivalve mollusks (oysters, clams, mussels, and scallops).

Bivalves are typically prepared by severing the adductor muscle, prying open the shell, and removing all of the soft tissue. The soft tissue includes viscera, meat, and body fluids.

11.2.4.3.2. Crabs

Edible tissue includes all leg and claw meat, back shell meat and body cavity meat. Internal organs generally are removed. If the crab is soft shelled, the entire crab is used in the sample.

11.2.4.3.3. Shrimp and Crayfish - Edible tissue includes the tail meat.

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11.2.4.3.4. Lobster - Edible tissue includes the tail and claw meat.

11.2.4.4. Worms

Samples are typically supplied to the lab in sample jars containing multiple organisms. Liquid and specimens constitute the entire sample and are blended together typically in the sample container. When a worm sample containing dirt particles or significant amounts of water is encountered, the technician should contact the project chemist to seek guidance from the client.

11.2.4.5. Eggs

Avian eggs are typically removed from the shell and blended. Aquatic eggs are blended including the soft shell.

11.2.4.6. Internal Organs Extraction

Organs such as livers or kidneys must be identified and removed by an experienced sample technician following clear written resection procedures or other guidance provided by the client.

11.2.4.7. Plant Tissue

Plant tissue should be handled using the size reduction, homogenization and implement cleaning steps outlined in Sections 11.2.1, 11.2.2, 11.2.3, and 11.2.4. Where these procedures are inappropriate, specific written procedures or guidance from the client is recommended.

If drying is requested by the client or is project-specified, a subsample for mercury analysis is taken from the wet sample, and then the plant tissue is dried at 60°C prior to homogenization.

11.2.4.8. Small Mammals and Rodents

11.2.4.8.1. There are two primary concerns in working with small mammals and rodents: safety and sample homogenization.

11.2.4.8.2. Small mammals are potential carriers of lethal viruses, such as hantavirus and rabies, and bacteria that can be contracted through inhalation or direct contact. Typically, these organisms are excreted in the feces and distributed on the air as the fecal matter dries. During the sample preparation process, tissue is typically freeze-dried in order to calculate a percent solids value and to analyze for metals. As such, it is possible to increase the potential for dispersion

of the bacteria or viruses after the sample is homogenized and processed. Prior to processing, all samples should be stored frozen.

11.2.4.8.3. Prior to sample homogenization, instructions should be received from the client regarding the processing of the hide. For organics, it is recommended that the hides be left on the carcass and the entire sample be homogenized. For metals, there is a potential for accumulation in the hair. As a non-digestible portion of the rodent, inclusion of the hair may result in a high bias if the data is to be used in estimating bioaccumulation up the food chain. Skinning may be a preferred alternative when metals are the primary chemicals of concern.

11.2.4.8.4. Homogenization should be done while the carcass is still partially frozen.

11.2.4.8.5. If the hide is to be included in the homogenization, snip the feet from the animal using stainless steel scissors.

11.2.4.8.5.1. The tail should be removed if it will prevent complete homogenization of the sample (e.g., the tail of a mouse or rat may result in incomplete homogenization and should not be included with the sample). Remove seeds, grasses, and mud from the hide.

11.2.4.8.6. If the hide is to be removed from the carcass, make an incision through the skin on the back of the neck (do not cut into the muscle). In most cases, the hide can be removed by pulling the incision horizontally along the back in one direction, and over the ears, head and snout in the opposite direction. The eyes are usually lost during this procedure. Continue to skin the animal by peeling the hide over the hind legs, off the underside of the animal, and around the front legs. The hide is removed at the hind legs and the snout. Care should be taken not to tear the connective tissue under the hide. Fat should be scraped from the hide when possible and included with the sample. Rinse the skinned carcass with DI water to remove any hair or dirt that has accumulated during the skinning procedure.

11.2.4.8.7. Homogenize the sample using a stainless steel Waring blender. Select a blender cup that is sized in accordance with the amount of sample to be homogenized. That is, small samples should be homogenized using small blender cups. This will improve the overall homogenization and recovery of the sample. Continue to mix the sample into a paste like consistency. Make sure no chunks of muscle, hide, or bone are distributed in the sample. Transfer the

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sample paste to a glass jar for storage and freeze. The new sample container is labeled “homogenized”, initialed, and dated.

11.2.5. General Provision for Handling Large Sample Mass

In some cases, large specimens will be received by the laboratory for homogenization prior to chemical analysis. For the purpose of this SOP, ‘large’ is defined as requiring preliminary size reduction to allow sequential processing of the sample. Sub-samples of the whole specimen should be cut to a size appropriate for the blender, mixer, or grinder that will be used. After each individual fraction is processed, the homogenized material is added to a reservoir large enough to hold all fractions as they accumulate. The reservoir will be constructed of a material suitable for the analytical application as defined under Section 10.2.3. For very large specimens (i.e. >20 pounds), high grade stainless steel containers are used (large bowls or small drums).

Blending of the combined fractions to achieve a whole homogenous material is achieved via manual mixing. In general, this is accomplished using a high grade stainless steel paddle or spoon of appropriate size (i.e. relative to the whole homogenate). Very large specimens (i.e. >20 pounds) generally require secondary processing through the grinder, particularly when large amounts of skin, bone, and/or cartilage is present. In these cases, the Hobart grinder is generally used.

11.3. Compositing

Each sample is to be logged in and receive a lab code. Additionally, the sample composite also is assigned a lab code. The compositing process is to be performed by trained staff. It is to be performed in an area free of contamination. It is imperative that the samples are treated in a manner consistent with the requirements of the tests to be performed on the composited sample. Compositing of homogenates should be performed according to this SOP or specific instructions provided by the client.

11.3.1. Documentation

The analyst preparing the composite will document

- that homogenization was done before removing an aliquot,
- the quantity of each (field or discrete) sample used for the composite,
- the date and time of compositing, and
- any other pertinent observations.
- An example of a data sheet that can be used for this purpose is given in Appendix C.

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11.3.2. Tissue Samples with Limited Quantity

Samples and sample composites that are quantity limited will be handled by the same procedure as described in Section 10.1.1

11.3.3. Compositing Procedure

11.3.3.1. Each tissue sample is first homogenized as per instructions in Section 11.2.

11.3.3.2. An equal weight of sample aliquot from each of the homogenized samples is weighed into a clean glass sample bottle. The amount to be weighed of each sample will depend upon the number of analyses to be performed on the composite and if the quantity of any individual sample is limited.

11.3.3.3. The mixture of the individual sample aliquots is thoroughly homogenized in the glass container. The composite sample bottle is labeled with:

- the name of the composite,
- the lab code of the composite,
- the analyst's initials
- the date of composite preparation
- The composite sample and the remaining (individual, discrete) samples are stored frozen until analysis.

11.3.4. Tissue Samples Requiring VOC Analyses

A separate aliquot of the composite homogenate should be placed in 40ml voa vial container for later analysis by the VOA department. Each container should be labelled with the lab identifier, date, initials, and "FOR VOA ANALYSIS ONLY". To minimize losses of volatile constituents, the sample should be kept as cold as possible, the work should be completed as quickly as possible, and the VOA vial filled to the top to minimize head space.

11.4. Subsampling

The sample is first thoroughly homogenized as per Section 11.2. A sample aliquot is removed and placed into a clean glass container of appropriate size and labeled as follows:

- the name of the sample,
- the lab code of the sample,
- "homogenized" written on the label,
- the purpose of the sub-sample (e.g. "dioxin subsample")
- the analyst's initials
- the date.

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11.5. Freeze-Drying

11.5.1. Depending on project specifications, samples may require freeze-drying. Freeze-drying may be performed on a separate portion of sample to determine % Freeze-Dried Solids, or may be done on the analytical subsample for certain tests. The analyst should obtain direction from the supervisor and/or Project Chemist.

11.5.2. Weigh 5-8 g of sample (wet weight) into a scintillation vial. Freeze the sample for at least 2 hours.

11.5.3. Remove the sample from the freezer and place in the freeze drier for at least 24 hours or longer if necessary for the particular sample matrix.

11.5.4. Record the measurements on the benchsheet in Appendix C.

12. QA/QC REQUIREMENTS

12.1. A rinsate blank should be prepared to accompany each batch of tissue samples. The blank is comprised of a collection of DI water rinses of cleaned equipment (knives, cutting boards and mixers/grinders) **prior** to the commencement of sample batch preparation. If contamination of the samples is suspected, the rinsate blank is extracted and analyzed for contaminants. The rinsate blank should be labeled with the extraction date and the associated SR numbers and stored at 4 C. In the event that contamination is suspected, the rinsate blank can be analyzed to confirm the presence of contaminants in the tissue preparation process.

12.2. A homogenization blanks are prepared to determine if the homogenization equipment was effectively cleaned between samples. Unless a project plan specifies otherwise, the laboratory prepares two homogenizations blanks with each shift of sample preparation. One is a 500 mL aliquot for Metals testing and the other is a 1000 mL aliquot for Organics testing. Any requirements other than the labs default procedure must be defined in the project plan and communicated to the laboratory.

12.2.1. Some project quality plans may require homogenization blanks between each sample. Following the blending of a tissue sample decontaminate the Hobart mixer (model HCM62) by following these steps:

- Wash the bowl, blade assembly, and lid with soap and hot water.
- Rinse all parts with dionized water.
- Move to fume hood and hexane rinse all parts.
- Allow excess hexane to evaporate.

12.2.2. Reassemble the mixer and make ready for the next sample.

12.2.3. Fill the bowl with dionized water and turn the mixer on for the approximately average time used for the type of samples being processed.

12.2.4. Aliquot the dionized water to bottles appropriate for the testing being conducted and preserve accordingly. If insufficient sample volume is produced for the required testing, repeat the procedure after the next tissue sample is homogenized.

13. RECORDS

Sample handling documentation must include information about sample homogenization (was it done or not), compositing, and subsampling. The data benchsheets in Appendix C provide a means for recording this information. This sample handling information is to be filed in the project file with the raw data.

14. CONTINGENCIES FOR HANDLING OUT-OF-CONTROL OR UNACCEPTABLE DATA

14.1. Refer to the SOP for Corrective Action (ADM-CA) for procedures for corrective action. Personnel at all levels and positions in the laboratory are to be alert to identifying problems and nonconformities when errors, deficiencies, or out-of-control situations are detected.

14.2. Handling out-of-control or unacceptable data

14.2.1. On-the-spot corrective actions that are routinely made by analysts and result in acceptable analyses should be documented as normal operating procedures, and no specific documentation need be made other than notations in laboratory maintenance logbooks, runlogs, for example.

14.2.2. Documentation of a nonconformity must be done using a Nonconformity and Corrective Action Report (NCAR) when:

- Corrective action is not taken or not possible
- Corrective action fails to correct an out-of-control problem on a laboratory QC or calibration analysis.
- Reanalysis corrects the nonconformity but is not a procedurally compliant analysis.

15. TRAINING

15.1. Training outline – Training Plan

15.1.1. Review literature (see references section). Read and understand the SOP. Also review the applicable MSDS for all reagents and standards used. Following these reviews, observe the procedure as performed by an experienced analyst at least three times.

15.1.2. The next training step is to assist in the procedure under the guidance of an experienced analyst until the supervisor feels lthe new employee can work independently. During this period, the analyst is expected to transition from a role

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of assisting, to performing the procedure with minimal oversight from an experienced analyst.

15.2. Training is documented following the SOP *for Documentation of Training*.

15.2.1. When the analyst training is documented by the supervisor on internal training documentation forms, the supervisor is acknowledging that the analyst has read and understands this SOP and that adequate training has been given to the analyst to competently perform the analysis independently.

16. REFERENCES

Kateman and L. Buydens, *Quality Control in Analytical Chemistry*, Second Edition, John Wiley & Sons, Inc., New York, NY, 1993: Chapter 2 on Sampling and especially sections 2.5 (Sample Quality) and 2.7 (Handling of Samples).

Guidance For Assessing Chemical Contaminant Data For Use In Fish Advisories; Volume 1; Fish Sampling and Analysis, 3rd Edition; USEPA Office of Water; EPA 823-B-00-007; Nov 2000.

Recommended Protocols for Measuring Selected Environmental Variables in Puget Sound; Tetra Tech, Inc.; final report TC-3991-04 Recommended Guidelines for Measuring Organic Compounds in Puget Sound Sediment and Tissue Samples Revision April 1996.

PCB's and Mirex In Fish Tissue and Clams New York State Department of Health Wadsworth Center For Laboratories and Research; Albany, N.Y. 10/6/81

Draft Method 1613-Tissue; Determination of PCDDs and PCDFs in Fish and Other Tissue Using Method 1613; USEPA Office of Water June 1993.

17. CHANGES SINCE THE LAST REVISION

17.1. Sec 7, 14 and 15 are new
17.2. Sec 8.1 is new
17.3. Sec 11.2.4.8.2 –removed last sentence describing irradiation procedure

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APPENDIX A

Fish Filleting Procedure

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APPENDIX B

General Procedure for Removing Edible Tissues From Shellfish

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APPENDIX C

Compositing and Freeze-Drying Bench Sheets

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COLUMBIA ANALYTICAL SERVICES, INC.

Service Request #: _____

Analysis For: Freeze Dried Solids

Lab Code	Wet Weight (g)	Tare (g)	Tare + Dry Wt.(g)	Dry Weight (g)	% Total Solids

Date/Time in Freeze Dryer:_____ Date/Time out of Freeze Dryer:_____

Balance ID:_____ Date Balance checked:_____

Comments:_____

_____

_____

_____

_____

x = RPD

Analyst:	Date:
Reviewed By:	Date:

Columbia Analytical Services, Inc.

Service Request Number(s):

TISSUE HOMOGENIZATION DATA

Laboratory ID	Weight of Sample* (g)	Tare of Jar (g)		Matrix	Number per Sample	

Comments:

* The weight of sample Is after homogenization.

Balance ID:	Date Balance Checked:
Analyst:	Date:
Reviewed:	Date:

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Service Request Number(s):

TISSUE WHOLE BODY WEIGHT, LENGTH, and HOMOGENIZATION

Laboratory ID	Weight of Sample* (g)	Tare of Jar (g)		Length of Sample (in)	Matrix	

Comments:

* The weight of sample is after homogenization.

Balance ID: Date Balance Checked:

Analyst:	Date:
Reviewed:	Date:

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Service Request Number(s):

Analysis for:
Pesticides PCB's Lipids SVO PAH Butyltins Dioxins SUB

ALIQUOT DATA

Service Request #		Wet Wt. (g)		Tare Wt. (g)		Matrix

Comments:

Balance ID: Date Balance Checked:

Analyst: Date:

Reviewed: Date:

Service Request Number(s):
Analysis for:

EQUIPMENT BLANKS

Service Request #		Bottle Type		Preservatives

Comments:	
Analyst:	DATE:
Reviewed:	

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Columbia Analytical Services, Inc.

Service Request #: _____

Analysis For: Freeze Dried Solids

Lab Code	Wet Weight (g)	Tare (g)	Tare + Dry Wt.(g)	Dry Weight (g)	% Total Solids
NRCC DORM-3	(If Applicable)				96.1%
NRCC TORT-2	(If Applicable)				94.7%

Date/Time in Freeze Dryer:_____ Date/Time out of Freeze Dryer:_____

Balance ID:_____ Date Balance checked:_____

Comments:_____

_____

_____

_____

_____

High - Low / Average = RPD

x = RPD

Analyst:	Date:
Reviewed By:	Date:

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STANDARD OPERATING PROCEDURE

ORGANOCHLORINE PESTICIDES BY GAS CHROMATOGRAPHY

SOC-8081
Revision 16
August 26, 2011

Approved By:

Supervisor		Date 8/3/11
QA Manager		Date 8/3/11
Laboratory Director		Date 8/3/11

COLUMBIA ANALYTICAL SERVICES, INC.
1317 South 13th Avenue
Kelso, Washington 98626

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DOCUMENT CONTROL	
NUMBER:	
Initials:	Date:

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1. SCOPE AND APPLICATION

This procedure is used to determine the concentrations of Organochlorine Pesticides in liquid and solid matrices using EPA Method 8081B. Table 1 indicates compounds that are routinely determined by this procedure and lists the method reporting limits (MRLs) in water and soil/sediment. Additional analytes may also be determined as required by specific projects. The reported MRL may be adjusted if required for specific project requirements; however, the capability of achieving other reported MRLs must be demonstrated. Method Detection Limits that have been achieved are given in Attachment A. MDLs may change as repeat studies are conducted. A low-level sediment option may be used, with MRLs given in Table 2.

2. SUMMARY OF METHOD

2.1. This procedure provides gas chromatographic conditions for the detection of low concentration (typically parts-per-billion level organochlorine pesticides) pesticides. Target analytes are extracted from the sample and isolated via extract cleanup if needed. Liquid samples are extracted using continuous liquid-liquid extraction (Method 3520, CAS SOP EXT-3520), or solid phase extraction (Method 3535, CAS SOP EXT-3535). TCLP leachates are extracted using separatory funnel (Method 3510, CAS SOP EXT-3510). In general, soil/sediment samples are extracted using Soxhlet (Method 3540, CAS SOP EXT-3540) or automated Soxhlet extraction (Method 3541, CAS SOP EXT-3541).

2.2. A portion of the extract is analyzed using a gas chromatograph (GC) equipped with dual column fused silica capillary columns and dual electron capture detectors (ECD). Identification is based on comparison of sample retention times to the retention times of known target compounds. Quantitative analysis is performed by using certified standards to produce a calibration curve response factor. Analyte concentration can then be calculated using theresponse factor of the calibration curve. Sample concentration is calculated using the extract concentration and the extracted sample weights, volumes and dilution factors.

2.3. The sensitivity of the procedure depends on the level of interferences rather than instrument limitations. If interferences prevent the detection of analytes, GPC cleanup, Florisil cleanup, sulfur cleanup, carbon cleanup, and other appropriate techniques may be used to reduce interferences.

2.4. In cases where there is a project-specific quality assurance plan (QAPP), the project manager identifies and communicates the QAPP-specific requirements to the laboratory. In general, project specific QAPP's supersede method specified requirements. An example of this are projects falling under DoD ELAP. QC requirements defined in the SOP *Department of Defense Projects – Laboratory Practices and Project Management (ADM-DOD)* may supersede the requirements defined in this SOP.

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3. DEFINITIONS

3.1. Analysis Sequence - Samples are analyzed in a set referred to as an analysis sequence. The sequence begins with instrument calibration followed by sample extracts interspersed with calibration standards. The sequence ends when the set of samples has been injected or when qualitative and/or quantitative QC criteria are exceeded. See the SOP *ADM-BATCH*.

3.2. Independent Calibration Verification (ICV) - Verification of the ratio of instrument response to analyte amount, a calibration check, is done by analyzing for analyte standards in an appropriate solvent. ICV solutions are made from a stock solution which is different from the stock used to prepare calibration standards.

3.3. Internal Standards - Internal standards are organic compounds which are similar to the analytes of interest but which are not found in the samples. The chosen internal standards are used to help calibrate the instrument's response and to compensate for slight instrument variations from injection to injection.

3.4. Matrix Spike/Duplicate Matrix Spike Analysis - In the matrix spike analysis, predetermined quantities of stock solutions of certain analytes are added to a sample matrix prior to sample extraction and analysis. The purpose of the matrix spike is to evaluate the effects of the sample matrix on the methods used for the analyses. Samples are split into duplicates, spiked, and analyzed. Percent recoveries are calculated for each of the analytes detected. The relative percent difference between the samples is calculated and used to assess analytical precision.

3.5. Standard Curve - A standard curve is a curve which plots concentrations of a known analyte standard versus the instrument response to the analyte.

3.6. Surrogate - Surrogates are organic compounds which are similar to analytes of interest in chemical composition, extraction, and chromatography, but which are not normally found in environmental samples. The purpose of the surrogates is to evaluate the preparation and analysis of samples. These compounds are spiked into all blanks, standards, samples, and spiked samples prior to analysis. Percent recoveries are calculated for each surrogate.

3.7. Method Blank - The method blank is an artificial sample designed to monitor introduction of artifacts into the process. The method blank is carried through the entire analytical procedure.

3.8. Continuing Calibration Verification Standard (CCV) - A standard injected into the instrument at specified intervals and is used to verify the initial calibration.

3.9. Instrument Blank (CCB) - The instrument blank (also called continuing calibration blank) is a volume of clean solvent analyzed on each GC column and instrument used for sample

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analysis. The purpose of the instrument blank is to determine the levels of contamination associated with the instrumental analysis itself, particularly with regard to the carry-over of analytes from standards or highly contaminated samples.

4. INTERFERENCES

4.1. Solvents, reagents, glassware, gases, and sample processing hardware may yield discrete artifacts and/or elevated baselines, causing misinterpretation of the chromatograms. All of these materials must be demonstrated to be free from interferences, under the conditions of the analysis, by running method blanks.

4.2. Interferences from phthalate esters introduced during sample handling can pose a problem with pesticide determinations. Analysts should take precautions not to introduce phthalates during the analysis and sample preparation process. Much interference can be removed using GPC (SOP *SOC-3640A*) and/or Florisil cleanup (SOP *EXT-FLOR*). Florisil cleanup is typically used to reduce matrix interferences caused by polar compounds. The presence of elemental sulfur will result in peaks interfering with early eluting pesticides. Cleanup via method 3660 (SOP *SOC-3660*) may be used for the removal of sulfur if GPC cleanup is inadequate. Other co-extractables such as lipids, waxes, etc., are removed via GPC cleanup. A Cleanup procedure using carbon cartridges (SOP *EXT-CARCU*) that eliminate compounds which interfere with gas chromatographic analyses may also be utilized.

4.3. xtracts using carbon cartridges. Carbon cleanup is a convenient way to eliminate compounds that interfere with gas chromatographic analysis of certain target analytes.

5. SAFETY

5.1. All appropriate safety precautions for handling solvents, reagents and samples must be taken when performing this procedure. This includes the use of personnel protective equipment, such as, safety glasses, lab coat and the correct gloves.

5.2. Chemicals, reagents and standards must be handled as described in the CAS safety policies, approved methods and in MSDSs where available. Refer to the CAS *Environmental, Health and Safety Manual* and the appropriate MSDS prior to beginning this method.

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6. SAMPLE COLLECTION, CONTAINERS, PRESERVATION, AND STORAGE

6.1. Containers used to collect samples should be purchased pre-cleaned containers. Alternatively, containers used to collect samples for the determination of semivolatile organic compounds may be soap and water washed followed by methanol (or isopropanol) rinsing. The sample containers should be of glass or Teflon and have screw-top covers with Teflon liners. In situations where Teflon is not available, solvent-rinsed aluminum foil may be used as a liner. Highly acidic or basic samples may react with the aluminum foil, causing eventual contamination of the sample. Plastic containers or lids may not be used for the storage of samples due to the possibility of sample contamination from the phthalate esters and other hydrocarbons within the plastic. Sampling should be performed according to SW-846 guidelines or project-specific procedures.

6.2. Water and soil samples must be iced or refrigerated at 4 ± 2°C from time of collection until extraction. Water samples must be extracted within 7 days of collection and soil/sediment samples must be extracted within 14 days of collection.

6.3. Sample extracts are stored at 4º in the dark and must be analyzed within 40 days.

7. STANDARDS, REAGENTS AND EQUIPMENT

7.1. Standards

7.1.1. The following commercially prepared stock standards and ICV stock standards are purchased from various vendors and must be certified by the manufacturer. The ICV stock standards are obtained from a different source from the initial calibration. The expiration date for unopened ampules is the manufacturer's assigned expiration date. If the manufacturer does not assign a date, use an expiration date of 1 year from receipt. Stock standards are stored at -10°C.

Standard	Concentration	Use
8081	1000 ug/mL	ICAL
8081 Surrogate[a]	200 ug/mL	ICAL
Isodrin	100 ug/mL	ICAL
Hexachlorobenzene	100 ug/mL	ICAL
8081 Misc. compounds*	250 ug/mL	ICAL
Chlordane	5000 ug/mL	ICAL
Toxaphene	1000 ug/mL	ICAL
8081	1000 ug/mL	ICV
Isodrin	100 ug/mL	ICV
Hexachlorobenzene	100 ug/mL	ICV
8081 Misc. compounds	100 ug/mL	ICV
Chlordane	1000 ug/mL	ICV
Toxaphene	1000 ug/mL	ICV
8081 Internal Standard[b]	5000ug/mL	Internal standard

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[a] Surrogates are Decachlorobiphenyl (DCB) and 2,4,5,6-Tetrachlorometaxylene (TCMX).
[b] Internal standard is 1-Bromo-2-nitrobenzene.

7.1.2. Intermediate standard solutions and ICV solutions are made in hexane by diluting stock standards to intermediate concentrations listed below. The ICV intermediates are prepared from the ICV stock standards obtained from a different source from the initial calibration stocks.

ICAL Intermediate	Concentration	Preparation
Combined 8081, isodrin, hexachlorobenzene, and surrogate.	10 ug/mL	1:100 dilution of 8081 stock, 1:10 dilution of isodrin & hexachlorobenzene stocks, 1:20 dilution of surr. stock.
8081 Misc. compounds*	10 ug/mL	1:25 dilution of stock
Chlordane	250 ug/mL	1:20 dilution of stock
Toxaphene	100 ug/mL	1:10 dilution of stock

Intermediate ICV Standard	Concentration	Preparation
Combined 8081, isodrin, and hexachlorobenzene	10 ug/mL	1:100 dilution of 8081 stock, 1:10 dilution of isodrin & hexachlorobenzene stocks.
8081 Misc. compounds*	10 ug/mL	1:10 dilution of stock
8081 Internal Stanard	5ug/mL	1:1000 dilution of stock

* Prepare only as needed for projects requiring non-routine additional compounds.

7.1.3. Working Standard Solutions

7.1.3.1. Calibration standards are prepared containing surrogates and analytes in hexane. Calibration standards are stored at 4º for up to six months. A series of standards are prepared from a common intermediate representing the MRL (or lower) to a value near the high end of the linear range. Calibration standards are prepared in hexane. See Table 4 for preparation and concentrations, including standards designated as CCVs.

7.1.3.2. The independent calibration verification (ICV) standards are prepared from stock solutions from a different source from the initial calibration as listed below. Expiration periods are the same as for equivalent stock and calibration standards.

ICV Standard	Concentration	Preparation
Combined 8081, isodrin, and hexachlorobenzene	40 ug/L	1:250 dilution of combined 8081 ICV intermediate.
8081 Misc. compounds	40 ug/L	1:250 dilution of 8081 misc. compounds intermediate.
Chlordane	800 ug/L	1:1250 dilution of Chlordane

		ICV stock.
Toxaphene	1000 ug/L	1:1000 dilution of Toxaphene ICV stock.

7.1.3.3. A surrogate spiking solution is prepared at 2ug/mL by making a 1:100 dilution of the surrogate stock standard in acetone. The surrogate solution is stored at 4º or up to six months.

7.1.3.4. An internal standard solution is prepared at 10ug/ml by diluting the internal stock standard in hexane. 5.0 uL of internal standard is added to each standard, blank and sample prior to analysis for a final concentration of 50ng/mL.

7.1.3.5. A matrix spike solution at 4ug/mL is prepared by diluting the stock solution in methanol. This solution is stored at 4º for 2 weeks. The matrix spike solution is added to all matrix spikes and lab control samples as outlined in section 12.

Spiking Solution	Concentration	Preparation
8081, isodrin, and hexachlorobenzene.	4 ug/mL	1:250 dilution of 8081 stock, 1:25 dilution of isodrin & hexachlorobenzene stocks.
8081 Misc. compounds*	4 ug/mL	1:25 dilution of stock
Chlordane**	10 ug/mL	1:500 dilution of stock
Toxaphene**	10 ug/mL	1:100 dilution of stock

* Prepare only as needed for projects requiring non-routine additional compounds.
** Prepare only as needed for project requirements.

7.1.4. Solvents: Hexane, acetone, methylene chloride, isooctane, and methanol. Pesticide grade or equivalent

8. APPARATUS AND EQUIPMENT

8.1. GC Instrumentation - Dual Column

8.1.1. The dual column approach involves a single injection split between two columns mounted in a single gas chromatograph (Hewlett Packard 5890, 6890, 7890 or equivalent) equipped with cool-on-column, split/splitless, or temperature programmable injection system; with dual ECDs. See Table 3 for typical chromatographic conditions. Helium or hydrogen can be used as the carrier gas. Argon/methane mixture or Nitrogen is used as the detector make-up gas. Current instrumental systems are identified as follows:

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Instrument I.D.	Analytical System	Routine Matrix
GC23	Agilent 6890	Water/Soil/Tissue
GC28	Agilent 6890	Water
GC34	Agilent 7890A	Water/Soil/Tissue

8.1.2. Columns, J&W columns typically are used;

Column 1 : DB-XLB 30m x 0.32mm ID, 0.50 µm df or equivalent*
Column 2 : DB-35MS 30m x 0.32mm ID, 0.25 µm df or equivalent*

Note: Column diameter and film thickness may vary depending on instrument. Refer to the instrument maintenance logbook for the columns used for a specific instrument configuration.

8.1.3. Autosampler, capable of reproducible injections, Hewlett Packard/Agilent 7673 or equivalent.

8.1.4. Data System - A computer data system must be interfaced to the GC/ECD. The system must allow the continuous acquisition and storage on machine-readable media of all chromatographic data obtained throughout the duration of the chromatographic program. The computer must have software that includes automated calibration, identification, and quantitation routines. The software must also be capable of integrating the chromatographic peaks abundances. The most recent version of the manufacturer's software is preferred. HP Enviroquant.

9. PREVENTIVE MAINTENANCE

9.1. All maintenance activities are recorded in a maintenance logbook kept for each instrument.

9.2. Carrier gas - Inline purifiers or scrubbers should be in place for all sources of carrier gas. These are selected to remove water, oxygen, and hydrocarbons. Purifiers should be changed as recommended by the supplier.

9.3. Gas Chromatograph

9.3.1. Whenever GC maintenance is performed, care should be taken to minimize the introduction of air or oxygen into the column. Injection port maintenance includes changing the injection port liner, seal, washer, o-ring, septum, column ferrule, and autosampler syringe as needed. Liners and seals should be changed when recent sample analyses predict a problem with chromatographic performance. In some cases liners and seals may be cleaned and re-used.

9.3.2. Clipping off a small portion of the head of the column often improves chromatographic performance. When cutting off any portion of the column, make sure the cut is straight and "clean" (uniform, without fragmentation) by using the proper column cutting tool. Clipping the column should be done from the guard column as the first option. If needed, replace the guard column. Only clip from the analytical column as a last option.

9.3.3. Over time, the column will exhibit poorer overall performance, as contaminated sample matrices are analyzed. The length of time for this to occur will depend on the samples analyzed. When a noticeable decrease in column performance is evident and other maintenance options do not result in improvement, the column should be replaced. This is especially true when evident in conjunction with calibration difficulties.

10. RESPONSIBILITIES

10.1. It is the responsibility of the analyst to perform the analysis according to this SOP and to complete all documentation required for data review. Analysis and interpretation of the results are performed by personnel in the laboratory who have demonstrated the ability to generate acceptable results utilizing this SOP. This demonstration is in accordance with the training program of the laboratory. Final review and sign-off of the data is performed by the department supervisor/manager or designee.

10.2. It is the responsibility of the department supervisor/manager to document analyst training. Documenting method proficiency, as described in 8081B, is also the responsibility of the department supervisor/manager.

11. PROCEDURE

11.1. Sample Preparation

11.1.1. Water samples (1L) are extracted at a pH of 5-9 with methylene chloride, using Method 3520 (SOP *EXT-3520)*, or solid phase extraction (SOP *EXT-3535*). TCLP leachates are extracted using separatory funnel (SOP *EXT-3510*). Refer to the applicable extraction SOP. For extraction by 3535, acidification of the sample prior to extraction may be allowable if project objectives and performance requirements of methods 3535 and 8081 are met.

Note: For projects originating from South Carolina and under the SC DHEC lab certification, use the 3535 extraction method only.

11.1.2. Soil/sediment samples are extracted using either EPA Method 3540 (SOP *EXT-3540*) or method 3541 (SOP *EXT-3541*). A low-level sediment option may be used where

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the sample weight or volume is adjusted to lower the level of detection in the sample. Refer to the applicable extraction SOP.

Note: For projects originating from South Carolina and under the SC DHEC lab certification, use the 3541 extraction method only.

11.1.3. Additional sample cleanup procedures may be used as appropriate for the samples. See Section 4.2 and refer to the applicable cleanup SOP.

11.2. Calibration

11.2.1. Refer to the SOP *Sample Batches* (*ADM-BATCH*) for guidance on analytical calibration and sample batches.

Note: Refer to the *SOP for Calibration of Instruments for Organics Chromatographic Analyses (SOC-CAL)*, where calibration procedures and policies are described. The calibration procedure(s) and options chosen must follow the SOP *SOC-CAL*.

.

11.2.2. Check for degradation of 4,4'-DDT and Endrin by injecting a standard containing only 4,4'-DDT at 100ppb and Endrin at 50 ppb.

$$\%\ Breakdown\ =\ \frac{Total\ DDT\ degratation\ peak\ area\ (DDE\ +\ DDD)}{Total\ DDT\ peak\ area\ (DDT\ +\ DDE\ +\ DDD)}\ x\ 100$$

$$\%\ Breakdown\ =\ \frac{\begin{array}{c}Total\ endrin\ degratation\ peak\ area\\ endrin\ aldehyde\ +\ endrin\ Ketone\end{array}}{\begin{array}{c}Total\ endrin\ peak\ area\\ endrin\ +\ endrin\ aldehyde\ +\ endrin\ Ketone\end{array}}\ x100$$

If degradation of either DDT or Endrin exceeds 15%, perform any necessary maintenance before proceeding with calibration. The breakdown of DDT and Endrin must be measured before samples are analyzed and at the beginning of each analytical sequence.

11.2.3. After determining that degradation is within acceptance, calibrate the system immediately prior to conducting any analyses. Analyze each calibration standard (containing internal standards) and tabulate the area against concentration for each compound. For multi-component analytes, only those specified in the project plan or work specification are used for calibration. Calculate response factors (RFs) for each compound relative to one of the internal standards as follows:

$$RF = (A_x C_{is})/(A_{is} C_x)$$

where:

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A_x = Area of the compound being measured.
A_{is} = Area of the specific internal standard.
C_{is} = Concentration of the specific internal standard (ng/µL).
C_x = Concentration of the compound being measured (ng/µL).

Note: For Chlordane, a minimum of 3 peaks must be chosen and for Toxaphene a minimum of 4 peaks must be chosen. The peaks must be characteristic of the compound of interest.

11.2.2.2 Calculate the mean response factor $(\overline{RF_x})$ for each analyte and surrogate from the calibration levels. Calculate standard deviation (SD) and the percent relative standard deviations (%RSD) for each analyte from the mean with:

$$\%RSD = \frac{(SD)}{(\overline{RF_x})} 100.$$

11.2.2.3 The % RSD should be less than 20% for each compound.

11.2.2.4 If the % RSD for any compound is 20% or less, linearity can be assumed over the calibration range, and the relative response factor for each analyte and surrogate is used to quantitate sample analytes.

11.2.2.5 If the %RSD exceeds 20%, then a linear curve or a quadratic calibration with a correlation coefficient of 0.990 or greater may be used.

11.2.4. Following initial calibration, analyze an ICV standard. The ICV solution must contain all analytes in the calibration standards. Calculate the concentration using the typical procedure used for quantitation. Calculate the percent difference (%D) from the ICV true value. Evaluate the ICV as described in the SOC-CAL SOP. The acceptance criterion is ± 20% from the analytes assigned value.

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11.2.5. Continuing Calibration Verification

11.2.5.1. The working calibration curve or calibration response factors must be verified on each analytical sequence by the analysis of one or more mid-range calibration standards (CCV). A CCV must be injected at the start of each 12 hour shift or every 20 samples, whichever is first. The 12 hour window starts with the injection time of the first CCV. The use of internal standard calibration technique does not require that all sample results be bracketed with calibration verification standards.

Note: DoD projects require a CCV analysis every 10 field samples.

11.2.5.2. The acceptance criteria for all analytes in the CCV analysis are a response (RF or concentration) within ± 20% D of the expected value, as compared to the initial calibration. Refer to the SOP *SOC-CAL*.

11.2.5.3. The measured area of the internal standard must be no more than -50% to + 100% difference from the average area calculated during initial calibration.

11.2.5.4. The retention time of the internal standard must also be evaluated. A retention time shift of >30 sec necessitates system maintenance and reanalysis of CCV.

11.2.6. Retention Time Windows

11.2.6.1. Establish retention time windows with the GC system in acceptable operating conditions. Make three injections of all analytes throughout the course of a 72-hour period. Serial injections over less than a 72-hour period may result in retention time windows that are too tight. Using retention times from these analyses, calculate retention time windows. Refer to EPA Method 8000 for detailed instructions.

11.2.6.2. Plus or minus three times the standard deviation of the absolute retention times for each standard will be used to define the retention time window; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms. In those cases where the standard deviation for a particular standard is zero, the laboratory may use a default window of ± 0.03 minutes. If the peak width is > 0.06 minutes, use a default window of 0.1 minutes.

11.2.6.3. Calculate retention time windows for each standard on each GC column and whenever a new GC column is installed. Retain this data in the method file.

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11.3. Sample Analysis

11.3.1. Table 3 indicates the typical operating conditions for the GC. Setup the analysis sequence of sample and QC samples. Also, refer to the SOP for *Sample Batches* for guidance.

11.3.2. Calibrate the system as described in Section 11.2. Evaluate the CCVs as discussed in Section 11.2.4. If any standard falls outside of their daily retention time window, evaluate the chromatogram for possible causes such as carryover from a highly contaminated sample. If a problem related to GC system has been determined to be the cause of retention time shift, perform whatever maintenance is necessary before re-injecting a CCV or recalibrating and proceeding with sample analysis.

11.3.3. Spike 5 ul of the internal standard 1-Bromo-2-nitrobenzene at 10 ppm into each 1 ml of sample extract to give a final concentration of 50ng/ml. The measured area of the internal standard must be -50 to +100% as measured from the average of the most recent calibration. Any samples falling outside of this criterion require reanalysis.

11.3.4. The retention time of the internal standard must also be evaluated. A retention time shift of >30 sec requires reanalysis of all affected samples.

11.4. Identification of Analytes

11.4.1. Tentative identification begins with a response that is >3 times the baseline noise level(s/n). The retention time of the peak is compared against the retention time of a known compound in the daily standard chromatogram.

11.4.2. Tentative identification of an analyte occurs when a peak from a sample extract falls within the daily retention time window and the s/n ratio of the peak is >3. A tentatively identified compound is confirmed when the retention time for the compound on the confirmatory detector is within the retention time window on that system.

11.4.3. Confirmation of all tentative hits should be made. Confirmation is made by injecting the sample extract on two columns with dissimilar phases simultaneously. If the retention time matches on both columns, then the hit for the analyte is considered a confirmed hit. Refer to the SOP for *Confirmation of Organic Analytes*.

11.4.4. For Chlordane a minimum of 3 peaks must be chosen, and for Toxaphene a minimum of 4 peaks must be chosen for identification purposes. Refer to Section 13 for quantitation procedures for multi-response analytes.

11.5. Perform all necessary calculations as described in Sections 12 and 13.

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12. QUALITY CONTROL

12.1. Initial Precision and Recovery Validation

The precision of the extraction procedure and the GC procedure must be validated before analysis of samples begins, or whenever significant changes to the procedures have been made. To do this, four reagent water samples are spiked at a level near t he midpoint of the calibration range (typically the LCS level), then extracting and proceeding with Section 11. The (spiking solution may be prepared from pure standard materials, or purchased as certified solutions. If prepared by the laboratory, stock standards prepared independently from those used for calibration should be used. The concentration of the analytes to be spiked is 20x the MRL.

12.2. Method Detection Limits

12.2.1. A method detection limit (MDL) study must be undertaken before analysis of samples can begin. Refer to the *SOP Performing Method Detection Limit Studies and Establishing Limits of Detection and Quantification.* The MDL study must be verified annually.

12.2.2. To establish detection limits that are precise and accurate, the analyst must perform the following procedure. Spike a minimum of seven blank replicates (i.e. 1L of reagent water for water MDLs, 10 or 20g sand for soil MDLs) with MDL spiking solution at a level below the MRL. Follow the procedures starting in Section 11 to analyze the samples.

12.3. Limits of Quantification (LOQ)

12.3.1. The laboratory must establish a LOQ for each analyte as the lowest reliable laboratory reporting concentration or in most cases the lowest point in the calibration curve which is less than or equal to the desired regulatory action levels, based on the stated project requirements. Analysis of a standard prepared at the LOQ concentration levels or use of the LOQs as the lowest point calibration standard provides confirmation of the established sensitivity of the method. The LOQ recoveries must be within 50% of the true values to verify the data reporting limit. Refer to the CAS SOP Performing Method Detection Limit Studies and Establishing Limits of Detection and Limits of Quantification.

12.4. Ongoing QC Samples required are described in the CAS-Kelso *Quality Assurance Manual* and in the *SOP for Sample Batches.* In general, these include:

12.4.1. Method blank - A method blank is extracted and analyzed with every batch of 20 or fewer samples to demonstrate that there are no method interferences. The method blank must demonstrate that interferences from the analytical and preparation steps are

minimized. No target analytes should be detected above the MRL in the method blank.

Note: DoD projects require that no analyte be detected > ½ the RL or 1/10 the regulatory limit, whichever is greater.

12.4.2. A lab control sample (LCS) must be extracted and analyzed with every batch of 20 samples. The water LCS is prepared by adding 50 µL of the matrix spike solution (250uL for Toxaphene/Chlordane) to 1L of reagent water, resulting in concentrations of 0.2 ug/L for single-response analytes (2.5 ug/L for Toxaphene or Chlordane). The soil LCS is prepared by adding 100 µL of spike solution (400uL for Toxaphene/Chlordane) to 20g of sand, resulting in concentrations of 20 ug/kg for single-response analytes (200 ug/kg for Toxaphene or Chlordane). For project-specific low-level extractions, spiking amounts can be adjusted accordingly.

$$\%R = X/TV \times 100$$

Where X = Concentration of the analyte recovered
TV = True value of amount spiked

Current CAS QC acceptance criteria for lab control samples are listed in Attachment A. Project-specific or program-specific acceptance criteria may supersede CAS criteria. For example, for samples requiring South Carolina DHEC certification the acceptance criteria are 70-130 % recovery. If the lab control sample (LCS) fails acceptance limits for any of the compounds, corrective action must be taken. Corrective action includes recalculation, reanalysis, or re-extraction and reanalysis. Refer to the *Quality Assurance Manual (QAM)* for guidance in evaluating recoveries that exceed LCS limits.

12.4.3. A matrix spike (MS) and duplicate matrix spike (DMS) must be extracted and analyzed with every batch of 20 samples. The MS/DMS is prepared by adding the same volume of the matrix spike solution to the sample as listed for the LCS, then proceeding with Section 11. Calculate percent recovery (%R) as:

$$\%R = \frac{X - X1}{TV} \times 100$$

Where X = Concentration of the analyte recovered
X1 = Concentration of unspiked analyte
TV = True value of amount spiked

Calculate Relative Percent Difference (RPD) as:

$$RPD = \frac{|R1 - R2|}{(R1 + R2)/2} \times 100$$

Where R1 = %recovery of the MS
R2 = %recovery of the DMS

Current CAS QC acceptance criteria for MS/DMS are listed in Attachment A. Project-specific acceptance criteria may supersede CAS criteria. If the MS/DMS recovery is out of acceptance limits for reasons other than matrix effects, corrective action must be taken. Corrective action includes recalculation, reanalysis, or re-extraction and reanalysis.

12.4.4. Surrogate spike is added to every sample, blank and spike prior to extraction. Two surrogate standards (tetrachloro-m-xylene and decachlorobiphenyl) are added to each sample. For water, 100µL of the surrogate spike is added to 1L, resulting in 0.2ug/L. For soil, 200µL of the surrogate spike is added to 20g, resulting in 20ug/kg. Calculate surrogate percent recovery (%R) as:

$$\%R = S/V \times 100$$

Where S = The amount of surrogate recovered
V = The amount spiked/final volume

12.5. Current CAS QC acceptance criteria for surrogates are listed in Attachment A. Project-specific acceptance criteria may supersede CAS criteria. Both surrogate recoveries must be within the acceptance limits. If either (or both) surrogate is outside of acceptance limits for reasons other than matrix interferences, corrective action must be taken. Corrective actions include recalculation, reanalysis, or re-extraction and reanalysis. The acceptance criteria listed are current criteria, but are subject to change as control limits are updated.

12.6. Additional QA/QC measures include control charting of QC sample results and periodic analysis of certified reference materials.

13. DATA REDUCTION, REVIEW, AND REPORTING

13.1. Both detectors are used as primary and/or confirmatory systems when not interfered with by the sample matrix.

13.2. Calculations

13.2.1. Quantitation of analytes in sample extracts is performed by comparing total area of residue peaks to total area or peaks from the appropriate reference materials.

13.2.2. Quantitation of multi-response analytes:

13.2.2.1. The quantitation of Chlordane, Toxaphene, and other multi-response analytes is accomplished by comparison of the sample chromatogram to that of the authentic standard. All calibration acceptance criteria as described in section 11 must be met before reporting any results. Sample results should then be reported according to the organics confirmation SOP (*SOC-CONF).* Results may be reported from either of the columns as long as all calibration acceptance criteria are met.

13.2.2.2. Once the analyte pattern has been identified, compare the responses of the major peaks in the calibration standard with the peaks observed in the sample extract. The amount of analyte is calculated using the individual calibration factor for each peak and the calibration model selected in section 11. The concentration is determined using the characteristic peaks and then the concentrations are averaged to determine the concentration. If there are interfering peaks that cause the average to be falsely overstated, then that interference peak is Q-deleted using the data system, provided 3 peaks remain for Chlordane and 4 peaks for Toxaphene. The average is then recalculated so that the average more truly represents the concentration in the sample.

The concentration of each analyte in the sample extract (Cex) is computed.. When $\overline{RF_x}$ is used, calculate the extract concentration as follows:

$$C_{ex} = \frac{(Resp_x)(Amt_{ISTD})}{(Resp_{ISTD})(\overline{RF_x})}$$

Where: C_{ex} = the concentration in the sample extract (ppm);
$Resp_x$ = the peak area of the analytes of interest;
$Resp_{ISTD}$ = the peak area of the associated internal standard;
Amt_{ISTD} = the amount, in ppm, of internal standard added
$\overline{RF_x}$ = the average response from the initial calibration.

13.2.3. The concentration of analytes in the original sample is computed using the following equations:

Aqueous Samples: $Concentration\ (\mu g / L) = \frac{(Cex)\ (Vf)\ (D)}{(Vs)}$

Where	Cex	=	Concentration in extract in µg/mL
	Vf	=	Final volume of extract in mL
	D	=	Dilution factor
	Vs	=	Volume of sample extracted, liters

Nonaqueous Samples: $Concentration\ (mg / Kg) = \frac{(Cex)\ (Vf)\ (D)}{(W)}$

Where	Cex	=	Concentration in extract in µg/mL
	Vf	=	Final volume of extract in mL
	D	=	Dilution factor
	W	=	Weight of sample extracted in grams.

13.3. Data Review

13.3.1. Following primary data interpretation and calculations, all data is reviewed by a secondary analyst. Following generation of the report, the report is also reviewed. Refer to the *SOP for Laboratory Data Review Process* for details. The person responsible for final review of the data report and/or data package should assess the overall validity and quality of the results and provide any appropriate comments and information to the Project Chemist to inclusion in the report narrative.

13.4. Reporting

13.4.1. Reports are generated using the STEALTH Data Reporting System which compiles the SMO login information. This compilation is then transferred to a file, which STEALTH uses to generate a report. The forms generated may be CAS standard reports, DOD, or client-specific reports. The compiled data from LIMS is also used to create EDDs.

13.4.2. As an alternative, reports are generated using Excel© templates located in R:\SVG\forms. The analyst should choose the appropriate form and QC pages to correspond to required tier level and deliverables requirements. The detected analytes surrogate and matrix spikes are then transferred, by hand or electronically, to the templates.

13.4.3. Sample concentrations are reported when all QC criteria for the analysis has been met or the results are qualified with an appropriate footnote. For Arizona projects the appropriate Arizona qualifier must be used.

14. CORRECTIVE ACTION

14.1. Refer to the SOP for *Corrective Action* for procedures for corrective action. Personnel at all levels and positions in the laboratory are to be alert to identifying problems and nonconformities when errors, deficiencies, or out-of-control situations are detected.

14.2. Handling out-of-control or unacceptable data

14.3. On-the-spot corrective actions that are routinely made by analysts and result in acceptable analyses should be documented as normal operating procedures, and no specific documentation need be made other than notations in laboratory maintenance logbooks, runlogs, for example.

14.4. Documentation of a nonconformity must be done using a Nonconformity and Corrective Action Report (NCAR) when:

- Corrective action is not taken or not possible
- Corrective action fails to correct an out-of-control problem on a laboratory QC or calibration analysis.
- Reanalysis corrects the nonconformity but is not a procedurally compliant analysis.

15. METHOD PERFORMANCE

15.1. This method was validated through single laboratory studies of accuracy and precision. Refer to the reference method for additional method performance data available.

15.2. The method detection limit (MDL) is established using the procedure described in the SOP for *Performing Method Detection Limit Studies and Establishing Limits of Detection and Quantification* (ADM-MDL). Method Reporting Limits are established for this method based on MDL studies and as specified in the CAS *Quality Assurance Manual (QAM).*

16. POLLUTION PREVENTION

It is the laboratory's practice to minimize the amount of solvents and reagents used to perform this method wherever technically sound, feasibly possible, and within method requirements. Standards are prepared in volumes consistent with laboratory use in order to minimize the volume of expired standards to be disposed of. The threat to the environment from solvents and/or reagents used in this method may be minimized when recycled or disposed of properly.

17. WASTE MANAGEMENT

The laboratory will comply with all Federal, State and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions as specified in the CAS EH&S Manual.

This method uses non-halogenated solvents and any waste generated from this solvent must be placed in the collection cans in the lab. The solvent will then be added to the hazardous waste storage area and disposed of in accordance with Federal and State regulations.

18. TRAINING OUTLINE

18.1. The following items provide guidelines for training analysts. Refer to the SOP *Documentation of Training (ADM-TRANDOC)* for further requirements.

18.1.1. Review applicable references listed in section 19 and this SOP. Review the MSDS for all chemicals used in the analysis.

18.1.2. Observe the procedure as performed by an experienced analyst at least three times.

18.1.3. Assist in the procedure under the guidance of an experienced analyst for at least one month, preferably three months. During this training period, the analyst is expected to progress from a role of assisting to a role of performing the procedure with minimal oversight.

18.1.4. Perform initial precision and recovery (IPR) study as described above for water samples. Summaries of the IPR are reviewed and signed by the supervisor. Copies may be forwarded to the employee's training file. For applicable tests, IPR studies should be performed in order to be equivalent to NELAC's Initial Demonstration of Capability.

18.2. Training is documented following the SOP for Documentation of Technical Personnel Training.

NOTE: When the analyst training is documented by the supervisor on internal training documentation forms, the supervisor is acknowledging that the analyst has read and understands this SOP and that adequate training has been given to the analyst to competently perform the analysis independently.

19. REFERENCES

19.1. EPA SW-846, Test Methods for Evaluating Solid Waste, Third Edition, Update III, December 1996, Method 8081A, Revision 1.

19.2. EPA SW-846, Determinative Chromatographic Separations, Update III, December 1996, Method 8000B, Revision 2.

19.3. EPA SW-846, Determinative Chromatographic Separations, On-Line, March 2003, Method 8000C, Revision 3.

19.4. *DoD Quality systems Manual for Environmental Laboratories* Version 4.2, 6/5/2003

19.5. 8000C Method criteria, Arizona DHS, 2/13/2007. Available online at http://www.azdhs.gov/lab/license/tech/8000cmethod.pdf

19.6. EPA SW-846, Method 8081B, *Organochlorine Pesticides by Gas Chromatography*, Revision 2, February, 2007.

20. CHANGES SINCE THE LAST REVISION

20.1. 7.1.3.4: Removed internal standard concentration at 5 µL and changed the amount added from 10.0 µL to 5 µL.
20.2. 8.1.1: Added GC34 to the list of current instruments.
20.3. 11.3.3: Changed the amount of internal standard from 10.0 µL to 5.0µL.
20.4. 11.3.3: Changed the amount of internal standard from 5 ppm to 10 ppm.
20.5. Section 19: Update references
20.6. Section4.2: Added CARCU to the paragraph.
20.7. Section 8.1: Added the option of using Hydrogen as a carrier gas.
20.8. 11.2.3: Updated section that replaces 1.0 µL with “an aliquot” of each calibration standard.

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TABLE 1 - CAS/KELSO DATA QUALITY OBJECTIVES

METHOD	ANALYTE	CAS No.	MATRIX	MDLa	MRL	LODb	LOQc	UNITS	Accuracy (LCS %Rec.)	Matrix Spike (%Rec.)	Precision (% RPD)
8081	2,4'-DDD	53-19-0	Soil	1.4	5	2.8	5	ug/kg	53-115	12-147	40
8081	2,4'-DDE	3424-82-6	Soil	0.94	5	1.9	5	ug/kg	49-112	24-141	40
8081	2,4'-DDT	789-02-6	Soil	0.5	5	1.9	5	ug/kg	44-120	15-141	40
8081	4,4'-DDD	72-54-8	Soil	1	5	3.3	5	ug/kg	46-146	19-143	40
8081	4,4'-DDE	72-55-9	Soil	0.11	5	3.3	5	ug/kg	46-141	22-142	40
8081	4,4'-DDT	50-29-3	Soil	0.85	5	3.3	5	ug/kg	46-151	19-154	40
8081	Aldrin	309-00-2	Soil	0.34	5	1.2	5	ug/kg	37-134	22-135	40
8081	alpha-BHC	319-84-6	Soil	0.35	5	1.2	5	ug/kg	36-139	23-133	40
8081	alpha-Chlordane	5103-71-9	Soil	1.2	5	3.3	5	ug/kg	41-134	24-132	40
8081	beta-BHC	319-85-7	Soil	0.5	5	1.2	5	ug/kg	38-142	22-142	40
8081	Chlordane	57-74-9	Soil	22	100	44	100	ug/kg	42-136	30-136	40
8081	Chlorpyrifos	2921-88-2	Soil	0.66	5	1.4	5	ug/kg	23-137	23-137	40
8081	cis-Nonachlor	5103-73-1	Soil	0.57	5	1.4	5	ug/kg	51-119	18-139	40
8081	delta-BHC	319-86-8	Soil	0.37	5	1.2	5	ug/kg	48-145	25-148	40
8081	Dieldrin	60-57-1	Soil	0.48	5	1.2	5	ug/kg	46-136	26-133	40
8081	Endosulfan I	959-98-8	Soil	1.7	5	3.3	5	ug/kg	35-121	15-119	40
8081	Endosulfan II	33213-65-9	Soil	0.86	5	3.3	5	ug/kg	39-128	13-129	40
8081	Endosulfan Sulfate	1031-07-8	Soil	0.57	5	1.2	5	ug/kg	43-138	20-134	40
8081	Endrin	72-20-8	Soil	0.45	5	1.2	5	ug/kg	40-152	22-145	40
8081	Endrin Aldehyde	7421-93-4	Soil	1.4	5	3.3	5	ug/kg	32-132	10-129	40
8081	Endrin Ketone	53494-70-5	Soil	0.55	5	1.2	5	ug/kg	47-135	19-139	40
8081	gamma-BHC (Lindane)	58-89-9	Soil	0.45	5	1.2	5	ug/kg	40-142	26-135	40
8081	gamma-Chlordane	5566-34-7	Soil	0.6	5	1.2	5	ug/kg	41-135	24-133	40
8081	Heptachlor	76-44-8	Soil	0.83	5	3.3	5	ug/kg	39-135	21-136	40
8081	Heptachlor Epoxide	1024-57-3	Soil	0.39	5	1.2	5	ug/kg	45-118	25-129	40
8081	Hexachlorobenzene	118-74-1	Soil	0.47	5	1.2	5	ug/kg	21-100	11-118	40
8081	Hexachlorobutadiene	87-68-3	Soil	0.53	5	1.4	5	ug/kg	31-109	10-107	40
8081	Hexachloroethane	67-72-1	Soil	0.47	5	1.4	5	ug/kg	29-103	10-92	40
8081	Isodrin	465-73-6	Soil	0.3	5	1.2	5	ug/kg	32-126	32-126	40
8081	Methoxychlor	72-43-5	Soil	0.61	5	1.2	5	ug/kg	42-147	24-151	40
8081	Mirex	2385-85-5	Soil	0.68	5	1.4	5	ug/kg	38-123	10-131	40

8081	Oxychlordane	27304-13-8	Soil	0.68	5	1.4	5	ug/kg	37-119	22-118	40
8081	Toxaphene	8001-35-2	Soil	37	250	74	250	ug/kg	44-137	20-155	40
8081	trans-Nonachlor	39765-80-5	Soil	0.53	5	1.4	5	ug/kg	39-123	24-120	40
8081	Decachlorobiphenyl (Surr.)	2051-24-3	Soil	NA	NA	NA	NA	%	22-130	NA	NA
8081	Tetrachloro-m-xylene (Surr.)	877-09-8	Soil	NA	NA	NA	NA	%	20-116	NA	NA
8081	2,4'-DDD	53-19-0	Water	0.00057	0.01	0.002	0.01	ug/L	51-112	51-112	30
8081	2,4'-DDE	3424-82-6	Water	0.00050	0.01	0.002	0.01	ug/L	29-126	29-126	30
8081	2,4'-DDT	789-02-6	Water	0.00059	0.01	0.002	0.01	ug/L	51-117	51-117	30
8081	4,4'-DDD	72-54-8	Water	0.0013	0.01	0.0045	0.01	ug/L	33-132	29-125	30
8081	4,4'-DDE	72-55-9	Water	0.00055	0.01	0.002	0.01	ug/L	41-116	24-129	30
8081	4,4'-DDT	50-29-3	Water	0.00039	0.01	0.001	0.01	ug/L	42-143	28-139	30
8081	Aldrin	309-00-2	Water	0.00063	0.01	0.002	0.01	ug/L	10-126	18-111	30
8081	alpha-BHC	319-84-6	Water	0.00061	0.01	0.002	0.01	ug/L	36-122	31-123	30
8081	alpha-Chlordane	5103-71-9	Water	0.00058	0.01	0.002	0.01	ug/L	45-115	34-120	30
8081	beta-BHC	319-85-7	Water	0.002	0.01	0.0045	0.01	ug/L	42-125	31-118	30
8081	Chlordane	57-74-9	Water	0.1	0.2	0.2	0.2	ug/L	45-148	45-148	30
8081	Chlorpyrifos	2921-88-2	Water	0.00083	0.01	0.002	0.01	ug/L	25-143	25-143	30
8081	cis-Nonachlor	5103-73-1	Water	0.00060	0.01	0.002	0.01	ug/L	42-125	42-125	30
8081	delta-BHC	319-86-8	Water	0.0017	0.01	0.0045	0.01	ug/L	48-123	40-129	30
8081	Dieldrin	60-57-1	Water	0.0022	0.01	0.0045	0.01	ug/L	50-115	32-121	30
8081	Endosulfan I	959-98-8	Water	0.00058	0.01	0.002	0.01	ug/L	35-115	17-118	30
8081	Endosulfan II	33213-65-9	Water	0.001	0.01	0.002	0.01	ug/L	40-127	19-122	30
8081	Endosulfan Sulfate	1031-07-8	Water	0.00068	0.01	0.002	0.01	ug/L	38-118	30-120	30
8081	Endrin	72-20-8	Water	0.00069	0.01	0.002	0.01	ug/L	48-126	34-133	30
8081	Endrin Aldehyde	7421-93-4	Water	0.0019	0.01	0.0045	0.01	ug/L	27-104	10-108	30
8081	Endrin Ketone	53494-70-5	Water	0.00082	0.01	0.002	0.01	ug/L	40-126	34-113	30
8081	gamma-BHC (Lindane)	58-89-9	Water	0.00084	0.01	0.002	0.01	ug/L	44-117	31-123	30
8081	gamma-Chlordane	5566-34-7	Water	0.002	0.01	0.0045	0.01	ug/L	47-113	35-119	30
8081	Heptachlor	76-44-8	Water	0.00036	0.01	0.001	0.01	ug/L	40-115	23-124	30
8081	Heptachlor Epoxide	1024-57-3	Water	0.00068	0.01	0.002	0.01	ug/L	49-109	28-122	30
8081	Hexachlorobenzene	118-74-1	Water	0.00053	0.01	0.002	0.01	ug/L	31-95	10-131	30
8081	Hexachlorobutadiene	87-68-3	Water	0.0019	0.01	0.0125	0.03	ug/L	10-95	10-95	30
8081	Hexachlorocyclopentadiene	77-47-4	Water	0.0011	0.01		0.01	ug/L	70-130	70-130	30
8081	Hexachloroethane	67-72-1	Water	0.0012	0.01	0.0125	0.03	ug/L	10-94	10-94	30
8081	Isodrin	465-73-6	Water	0.0007	0.01	0.002	0.01	ug/L	10-112	10-112	30

8081	Methoxychlor	72-43-5	Water	0.001	0.01	0.002	0.01	ug/L	43-143	30-137	30
8081	Mirex	2385-85-5	Water	0.00081	0.01	0.002	0.01	ug/L	43-126	43-126	30
8081	Oxychlordane	27304-13-8	Water	0.0010	0.01	0.002	0.01	ug/L	40-126	40-16	30
8081	Toxaphene	8001-35-2	Water	0.2	0.5	0.4	0.5	ug/L	36-137	28-135	30
8081	trans-Nonachlor	39765-80-5	Water	0.00092	0.01	0.002	0.01	ug/L	47-123	47-123	30
8081	Decachlorobiphenyl (Surr.)	2051-24-3	Water	NA	NA	NA	NA	%	35-128	NA	NA
8081	Tetrachloro-m-xylene (Surr.)	877-09-8	Water	NA	NA	NA	NA	%	20-102	NA	NA
8081	2,4'-DDD	53-19-0	Tissue	0.38	1.0			ug/Kg	43-117	43-117	40
8081	2,4'-DDE	3424-82-6	Tissue	0.21	1.0			ug/Kg	44-115	44-115	40
8081	2,4'-DDT	789-02-6	Tissue	0.12	1.0			ug/Kg	42-118	42-118	40
8081	4,4'-DDD	72-54-8	Tissue	0.41	1.0			ug/Kg	33-138	35-126	40
8081	4,4'-DDE	72-55-9	Tissue	1.0	1.0			ug/Kg	36-139	33-134	40
8081	4,4'-DDT	50-29-3	Tissue	1.0	1.0			ug/Kg	49-136	26-133	40
8081	Aldrin	309-00-2	Tissue	0.19	1.0			ug/Kg	46-116	38-116	40
8081	alpha-BHC	319-84-6	Tissue	0.12	1.0			ug/Kg	42-124	31-118	40
8081	alpha-Chlordane	5103-71-9	Tissue	0.24	1.0			ug/Kg	40-121	39-113	40
8081	beta-BHC	319-85-7	Tissue	0.53	1.0			ug/Kg	39-122	22-123	40
8081	Chlordane	57-74-9	Tissue	3.1	10			ug/Kg	44-114	44-114	40
8081	Chlorpyrifos	2921-88-2	Tissue	0.26	1.0			ug/Kg	10-123	10-123	40
8081	cis-Nonachlor	5103-73-1	Tissue	0.22	1.0			ug/Kg	16-134	16-134	40
8081	delta-BHC	319-86-8	Tissue	0.18	1.0			ug/Kg	44-141	38-133	40
8081	Dieldrin	60-57-1	Tissue	0.23	1.0			ug/Kg	40-120	33-120	40
8081	Endosulfan I	959-98-8	Tissue	0.28	1.0			ug/Kg	23-116	22-113	40
8081	Endosulfan II	33213-65-9	Tissue	0.30	1.0			ug/Kg	35-111	28-120	40
8081	Endosulfan Sulfate	1031-07-8	Tissue	0.25	1.0			ug/Kg	36-127	23-128	40
8081	Endrin	72-20-8	Tissue	0.21	1.0			ug/Kg	44-130	43-124	40
8081	Endrin Aldehyde	7421-93-4	Tissue	0.40	1.0			ug/Kg	11-110	10-118	40
8081	Endrin Ketone	53494-70-5	Tissue	0.34	1.0			ug/Kg	29-133	27-128	40
8081	gamma-BHC (Lindane)	58-89-9	Tissue	0.21	1.0			ug/Kg	44-123	34-123	40
8081	gamma-Chlordane	5103-74-2	Tissue	0.20	1.0			ug/Kg	38-121	34-123	40
8081	Heptachlor	76-44-8	Tissue	0.13	1.0			ug/Kg	39-126	37-122	40
8081	Heptachlor Epoxide	1024-57-3	Tissue	0.28	1.0			ug/Kg	43-119	39-113	40
8081	Hexachlorobenzene	118-74-1	Tissue	0.15	1.0			ug/Kg	10-113	35-111	40
8081	Hexachlorobutadiene	87-68-3	Tissue	0.34	1.0			ug/Kg	10-130	10-130	40
8081	Hexachloroethane	67-72-1	Tissue	0.17	1.0			ug/Kg	10-113	10-113	40

8081	Isodrin	465-73-6	Tissue	0.18	1.0		ug/Kg	13-133	13-133	40
8081	Methoxychlor	72-43-5	Tissue	0.61	1.0		ug/Kg	37-144	27-148	40
8081	Mirex	2385-85-5	Tissue	0.24	1.0		ug/Kg	15-127	15-127	40
8081	Oxychlordane	27304-13-8	Tissue	0.29	1.0		ug/Kg	15-128	15-128	40
8081	Toxaphene	8001-35-2	Tissue	9.3	50		ug/Kg	21-152	21-152	40
8081	trans-Nonachlor	39765-80-5	Tissue	0.24	1.0		ug/Kg	12-139	12-139	40
8081	Decachlorobiphenyl (Surr.)	2051-24-3	Tissue	NA	NA		%	22-121	-	-
8081	Tetrachloro-m-xylene (Surr.)	877-09-8	Tissue	NA	NA		%	29-117	-	-

a Method Detection Limits are subject to change as new MDL studies are completed.

a MDL is the smallest analyte concentration that can be demonstrated to be different from zero with 99% confidence

b The LOD is the smallest amount of a substance that must be present in a sample in order to be detected with 99% conficence.

Verification is acceptable if the response is > 3x instrument noise & 2nd column confirmed or pattern.

TABLE 2 - CAS/KELSO DATA QUALITY OBJECTIVES

METHOD	ANALYTE	CAS No.	MATRIX	MDLa	MRL	LODb	LOQc	UNITS	Accuracy (LCS %Rec.)	Matrix Spike (%Rec.)	Precision (% RPD)
8081 LL	2,4'-DDD	53-19-0	Soil	0.13	1.0	0.325	1.0	ug/kg	53-115	12-147	40
8081 LL	2,4'-DDE	3424-82-6	Soil	0.16	1.0	0.325	1.0	ug/kg	49-112	24-141	40
8081 LL	2,4'-DDT	789-02-6	Soil	0.058	1.0	0.125	1.0	ug/kg	44-120	15-141	40
8081 LL	4,4'-DDD	72-54-8	Soil	0.11	1.0	0.25	1.0	ug/kg	46-146	19-143	40
8081 LL	4,4'-DDE	72-55-9	Soil	0.11	1.0	0.25	1.0	ug/kg	46-141	22-142	40
8081 LL	4,4'-DDT	50-29-3	Soil	0.17	1.0	0.4	1.0	ug/kg	46-151	19-154	40
8081 LL	Aldrin	309-00-2	Soil	0.16	1.0	0.4	1.0	ug/kg	37-134	22-135	40
8081 LL	alpha-BHC	319-84-6	Soil	0.11	1.0	0.25	1.0	ug/kg	36-139	23-133	40
8081 LL	alpha-Chlordane	5103-71-9	Soil	0.1	1.0	0.25	1.0	ug/kg	41-134	24-132	40
8081 LL	beta-BHC	319-85-7	Soil	0.18	1.0	0.4	1.0	ug/kg	38-142	22-142	40
8081 LL	Chlordane	57-74-9	Soil	1.9	10	4.9	10	ug/kg	42-136	30-136	40
8081 LL	Chlorpyrifos	2921-88-2	Soil	0.15	1.0	0.3	1.0	ug/kg	23-137	23-137	40
8081 LL	cis-Nonachlor	5103-73-1	Soil	0.12	1.0	0.3	1.0	ug/kg	51-119	18-139	40
8081 LL	delta-BHC	319-86-8	Soil	0.074	1.0	0.25	1.0	ug/kg	48-145	25-148	40
8081 LL	Dieldrin	60-57-1	Soil	0.14	1.0	0.4	1.0	ug/kg	46-136	26-133	40
8081 LL	Endosulfan I	959-98-8	Soil	0.063	1.0	0.25	1.0	ug/kg	35-121	15-119	40
8081 LL	Endosulfan II	33213-65-9	Soil	0.14	1.0	0.4	1.0	ug/kg	39-128	13-129	40
8081 LL	Endosulfan Sulfate	1031-07-8	Soil	0.11	1.0	0.25	1.0	ug/kg	43-138	20-134	40
8081 LL	Endrin	72-20-8	Soil	0.094	1.0	0.25	1.0	ug/kg	40-152	22-145	40
8081 LL	Endrin Aldehyde	7421-93-4	Soil	0.12	1.0	0.25	1.0	ug/kg	32-132	10-129	40
8081 LL	Endrin Ketone	53494-70-5	Soil	0.093	1.0	0.25	1.0	ug/kg	47-135	19-139	40
8081 LL	gamma-BHC (Lindane)	58-89-9	Soil	0.08	1.0	0.25	1.0	ug/kg	40-142	26-135	40
8081 LL	gamma-Chlordane	5566-34-7	Soil	0.09	1.0	0.25	1.0	ug/kg	41-135	24-133	40
8081 LL	Heptachlor	76-44-8	Soil	0.12	1.0	0.25	1.0	ug/kg	39-135	21-136	40
8081 LL	Heptachlor Epoxide	1024-57-3	Soil	0.084	1.0	0.25	1.0	ug/kg	45-118	25-129	40
8081 LL	Hexachlorobenzene	118-74-1	Soil	0.2	1.0	0.4	1.0	ug/kg	21-100	11-118	40
8081 LL	Hexachlorobutadiene	87-68-3	Soil	0.21	1.0	0.675	1.0	ug/kg	31-109	10-107	40
8081 LL	Hexachlorocyclopentadiene	77-47-4	Soil	1	1.0	-	-	ug/kg	70-130	70-130	40
8081 LL	Hexachloroethane	67-72-1	Soil	0.33	1.0	0.675	1.0	ug/kg	29-103	10-92	40
8081 LL	Isodrin	465-73-6	Soil	0.17	1.0	0.4	1.0	ug/kg	32-126	32-126	40
8081 LL	Methoxychlor	72-43-5	Soil	0.19	1.0	0.4	1.0	ug/kg	42-147	24-151	40

8081 LL	Mirex	2385-85-5	Soil	0.099	1.0	0.3	1.0	ug/kg	38-123	10-131	40
8081 LL	Oxychlordane	27304-13-8	Soil	0.085	1.0	0.3	1.0	ug/kg	37-119	22-118	40
8081 LL	Toxaphene	8001-35-2	Soil	4.8	50	9.75	50	ug/kg	44-137	20-155	40
8081 LL	trans-Nonachlor	39765-80-5	Soil	0.087	1.0	0.3	1.0	ug/kg	39-123	24-120	40
8081 LL	Decachlorobiphenyl (Surr.)	2051-24-3	Soil	NA	NA	NA	NA	%	22-130	NA	NA
8081 LL	Tetrachloro-m-xylene (Surr.)	877-09-8	Soil	NA	NA	NA	NA	%	20-116	NA	NA
8081 LL	2,4'-DDD	53-19-0	Water	0.07	0.5	0.25	0.5	ng/L	51-112	51-112	30
8081 LL	2,4'-DDE	3424-82-6	Water	0.25	0.5	0.25	0.5	ng/L	29-126	29-126	30
8081 LL	2,4'-DDT	789-02-6	Water	0.13	0.5	0.25	0.5	ng/L	51-117	51-117	30
8081 LL	4,4'-DDD	72-54-8	Water	0.21	0.5	0.5	0.5	ng/L	33-132	29-125	30
8081 LL	4,4'-DDE	72-55-9	Water	0.19	0.5	0.5	0.5	ng/L	41-116	24-129	30
8081 LL	4,4'-DDT	50-29-3	Water	0.17	0.5	0.5	0.5	ng/L	42-143	28-139	30
8081 LL	Aldrin	309-00-2	Water	0.33	0.5	0.5	0.5	ng/L	10-126	18-111	30
8081 LL	alpha-BHC	319-84-6	Water	0.21	0.5	0.5	0.5	ng/L	36-122	31-123	30
8081 LL	alpha-Chlordane	5103-71-9	Water	0.27	0.5	0.5	0.5	ng/L	45-115	34-120	30
8081 LL	beta-BHC	319-85-7	Water	0.41	0.5	0.5	0.5	ng/L	42-125	31-118	30
8081 LL	Chlordane	57-74-9	Water	3	10	5	10	ng/L	45-148	45-148	30
8081 LL	Chlorpyrifos	2921-88-2	Water	0.23	1	0.4	1	ng/L	25-143	25-143	30
8081 LL	cis-Nonachlor	5103-73-1	Water	0.14	0.5	0.4	0.5	ng/L	42-125	42-125	30
8081 LL	delta-BHC	319-86-8	Water	0.14	0.5	0.4	0.5	ng/L	48-123	40-129	30
8081 LL	Dieldrin	60-57-1	Water	0.37	0.5	0.5	0.5	ng/L	50-115	32-121	30
8081 LL	Endosulfan I	959-98-8	Water	0.25	0.5	0.5	0.5	ng/L	35-115	17-118	30
8081 LL	Endosulfan II	33213-65-9	Water	0.35	0.5	0.5	0.5	ng/L	40-127	19-122	30
8081 LL	Endosulfan Sulfate	1031-07-8	Water	0.28	0.5	0.5	0.5	ng/L	38-118	30-120	30
8081 LL	Endrin	72-20-8	Water	0.49	0.5	0.5	0.5	ng/L	48-126	34-133	30
8081 LL	Endrin Aldehyde	7421-93-4	Water	0.21	0.5	0.4	0.5	ng/L	27-104	10-108	30
8081 LL	Endrin Ketone	53494-70-5	Water	0.32	0.5	0.5	0.5	ng/L	40-126	34-113	30
8081 LL	gamma-BHC (Lindane)	58-89-9	Water	0.47	0.5	0.5	0.5	ng/L	44-117	31-123	30
8081 LL	gamma-Chlordane	5566-34-7	Water	0.31	0.5	0.5	0.5	ng/L	47-113	35-119	30
8081 LL	Heptachlor	76-44-8	Water	0.18	0.5	0.4	0.5	ng/L	40-115	23-124	30
8081 LL	Heptachlor Epoxide	1024-57-3	Water	0.21	0.5	0.4	0.5	ng/L	49-109	28-122	30
8081 LL	Hexachlorobenzene	118-74-1	Water	0.27	0.5	0.5	0.5	ng/L	31-95	10-131	30
8081 LL	Hexachlorobutadiene	87-68-3	Water	0.095	0.5	0.25	0.5	ng/L	10-95	10-95	30
8081 LL	Hexachloroethane	67-72-1	Water	0.08	0.5	0.25	0.75	ng/L	10-94	10-94	30
8081 LL	Isodrin	465-73-6	Water	0.15	0.5	0.4	0.5	ng/L	10-112	10-112	30

8081 LL	Methoxychlor	72-43-5	Water	0.44	0.5	1	1	ng/L	43-143	30-137	30
8081 LL	Mirex	2385-85-5	Water	0.19	0.5	0.4	1.2	ng/L	43-126	43-126	30
8081 LL	Oxychlordane	27304-13-8	Water	0.069	0.5	0.25	0.5	ng/L	40-126	40-16	30
8081 LL	Toxaphene	8001-35-2	Water	17	25	18	25	ng/L	36-137	28-135	30
8081 LL	trans-Nonachlor	39765-80-5	Water	0.11	0.5	0.4	0.5	ng/L	47-123	47-123	30
8081 LL	Decachlorobiphenyl (Surr.)	2051-24-3	Water	NA	NA	NA	NA	%	35-128	NA	NA
8081 LL	Tetrachloro-m-xylene (Surr.)	877-09-8	Water	NA	NA	NA	NA	%	20-102	NA	NA

a Method Detection Limits are subject to change as new MDL studies are completed.

a MDL is the smallest analyte concentration that can be demonstrated to be different from zero with 99% confidence

b The LOD is the smallest amount of a substance that must be present in a sample in order to be detected with 99% conficence.

Verification is acceptable if the response is > 3x instrument noise & 2nd column confirmed or pattern.

TABLE 3

Gas Chromatograph Operating Conditions*

Gas Chromatograph:	Agilent 6890
Injection Port Temperature:	40°C, 40-250°C at 250°C/min., hold for 15 min., 250-325°C at 250°C/min., hold for 5 min.
Oven Temperature Program:	50°C hold for 0.5min., 50-150°C at 40°C/min.; Ramp 13°C/min. to 320°C, hold for 3.92 min.
Detector Temperature:	325°C
Injection Volume:	1 µL (5 µL for ultra low level analysis)
Column 1:	30-m, 0.32mm id, DB-XLB, 0.50µm film thickness or equivalent
Column 2:	30-m, 0.32mm id, DB-35MS, 0.25µm film thickness or equivalent.
Carrier Gas:	Helium
Auxillary Gas:	Argon/Methane
Data System:	HP Enviroquant

*The above instrument temperatures may be modified when determining additional single response or multi-response pesticides.

TABLE 4

CALIBRATION STANDARD PREPARATION

Initial Calibration Standards*

8081 Intermediate Std.	Initial Concentration	Final Volume	Solvent	Final Concentration	
10 uL	10 ug/mL	50 mL	Hexane	2 ug/L	
25 uL	↓	50 mL	↓	5 ug/L	
100 uL		50 mL		20 ug/L	
250 uL		50 mL		50 ug/L	CCV Standard
500 uL		50 mL		100 ug/L	
1.0 mL		50 mL		200 ug/L	
Chlordane Intermediate Std.	Initial Concentration	Final Volume	Solvent	Final Concentration	
5 uL	250 mg/L	50 mL	Hexane	25 ug/L	
10 uL	↓	↓	↓	50 ug/L	
20 uL				100 ug/L	
100 uL				500 ug/L	CCV Standard
200 uL				1000 ug/L	
400 uL				2000 ug/L	
Toxaphene Intermediate Std.	Initial Concentration	Final Volume	Solvent	Final Concentration	
50 uL	100 mg/L	50 mL	Hexane	100 ug/L	
125 uL	↓	50 mL	↓	250 ug/L	
250 uL		50 mL		500 ug/L	
500 uL		50 mL		1000 ug/L	CCV Standard
500 uL		25 mL		2000 ug/L	
1250 uL		25 mL		5000 ug/L	

* As needed for projects requiring non-routine additional compounds, similar dilutions are prepared to obtain calibration standards for these compounds.

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STANDARD OPERATING PROCEDURE

PCBS AS AROCLORS

SOC-8082Ar
Revision 15

Effective Date: November 7, 2011

Approved By:

Supervisor	10/11/11 Date
QA Manager	10/11/11 Date
Laboratory Director	10/11/11 Date

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PCBS AS AROCLORS

1. SCOPE AND APPLICATION

This procedure is used to determine the concentrations of PCBs as Aroclors using EPA Method 8082A. This procedure is typically applied to water, sediment, and soil matrices but may also be applicable to tissue or various miscellaneous waste samples. Table 1 lists the analytes that are determined by this procedure and lists the method reporting limits (MRLs) for each compound in water and soil. Equivalent nomenclature for MRL includes Estimated Quantitation Limit (EQL). Therefore, MRL=EQL. The reported MRL may be adjusted if required for specific project requirements; however, the capability of achieving other reported MRLs must be demonstrated. Method Detection Limits that have been achieved are given in Table 1. MDLs may change as repeat studies are conducted.

2. METHOD SUMMARY

2.1. This procedure provides gas chromatographic conditions for the detection of parts-per-billion (ppb) levels of PCBs. The target PCBs are extracted from samples using the appropriate procedure for the sample matrix (see applicable SOP), analyzed, and reported as Aroclors. Liquid samples are extracted using solid phase extraction (Method 3535, CAS SOP EXT-3535). Liquid samples containing solid material may be extracted by continuous liquid-liquid extraction (Method 3520, CAS SOP EXT-3520). Soil/sediment samples are extracted using Soxhlet (Method 3540, CAS SOP EXT-3540), automated Soxhlet extraction (Method 3541, CAS SOP EXT-3541) or by Ultrasonic extraction (Method 3550, CAS SOP EXT-3550). An aliquot of the extract is injected into the gas chromatograph (GC). The compounds are separated on a fused silica capillary column. Compounds of interest are detected by an electron capture detector. Identification of the analytes of interest is performed by comparing the retention times of the analytes with the respective retention times of an authentic standard and by comparison of elution patterns to those of Aroclor standards. Quantitative analysis is performed by using the authentic standard to produce a calibration factor or calibration curve, and using the calibration data to determine the concentration of an analyte in the extract. The concentration in the sample is calculated using the sample weight or volume and the extract volume.

2.2. The sensitivity of this method usually depends on the level of interferences rather than on instrument limitations. If interferences prevent detection of the analytes, GPC, florisil column cleanup, sulfur cleanup, or concentrated sulfuric acid cleanups are used to eliminate interferences in the analysis. Refer to section 4.2 for cleanup procedure references.

2.3. In cases where there is a project-specific quality assurance plan (QAPP), the project manager identifies and communicates the QAPP-specific requirements to the laboratory. In general, project specific QAPP's supersede method specified requirements. An example of this are projects falling under DoD ELAP. QC requirements defined in the SOP *Department of*

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Defense Projects – Laboratory Practices and Project Management (ADM-DOD) may supersede the requirements defined in this SOP.

3. DEFINITIONS

Analysis Sequence - Samples are analyzed in a set referred to as an analysis sequence. The sequence begins with instrument calibration followed by sample extracts interspersed with calibration standards. The sequence ends when the set of samples has been injected or when qualitative and/or quantitative QC criteria are exceeded.

Initial (or Independent) Calibration Verification (ICV) - Initial calibration verification standards which are analyzed after initial calibration with newly prepared standards but prior to sample analysis, in order to verify the validity of the standards used in calibration. Once it is determined there is no systematic error in preparation of the calibration standards, they are considered valid for subsequent calibrations (as methods and expiration dates allow). The ICV standards are prepared from a materials obtained from a source different from that used to prepare calibration standards.

Matrix Spike/Duplicate Matrix Spike Analysis - In the matrix spike analysis, predetermined quantities of stock solutions of selected Aroclors are added to a sample matrix prior to sample extraction and analysis. The purpose of the matrix spike is to evaluate the effects of the sample matrix on the methods used for the analyses. Samples are split into duplicates, spiked, and analyzed. Percent recoveries are calculated for each of the analytes detected. The relative percent difference between the samples is calculated and used to assess analytical precision. The concentration of the spike should be at 5 to 10 times the MRL or at levels specified by a project analysis plan.

Standard Curve - A standard curve is a calibration curve which plots concentrations of a known analyte standard versus the instrument response to the analyte.

Surrogate - Surrogates are organic compounds which are similar to analytes of interest in chemical composition, extraction, and chromatography, but which are not normally found in environmental samples. The purpose of the surrogates is to evaluate the preparation and analysis of samples. These compounds are spiked into all blanks, standards, samples, and spiked samples prior to analysis. Percent recoveries are calculated for each surrogate.

Method Blank - The method blank is an artificial sample designed to monitor introduction of artifacts into the process. The method blank is carried through the entire analytical procedure.

Continuing Calibration Verification Standard (CCV) - A standard injected into the instrument at specified intervals and is used to verify the initial calibration.

Instrument Blank (CCB) - The instrument blank (also called continuing calibration blank) is a volume of clean solvent analyzed on each GC column and instrument used for sample analysis. The purpose of the instrument blank is to determine the levels of contamination associated with the

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instrumental analysis itself, particularly with regard to the carry-over of analytes from standards or highly contaminated samples into other analyses.

Laboratory Control Samples (LCS) – The LCS is an aliquot of analyte free water or analyte free solid to which known amounts target analytes are added. The LCS is prepared and analyzed in exactly the same manner as the samples. The percent recovery is compared to established limits and assists in determining whether the batch is in control.

4. INTERFERENCES

4.1. Interferences by phthalate esters can pose a major problem in PCB determinations when using the electron capture detector. These compounds generally appear in the chromatogram as large, late-eluting peaks, especially in the 15% and 50% fractions from the florisil cleanup. Common flexible plastics contain varying amounts of phthalates. These phthalates are easily extracted or leached from such materials during laboratory operations. Phthalate contamination is not usually a problem in our laboratory operation.

4.2. Co-extractables such as lipids, waxes, etc., can be removed via GPC cleanup (CAS SOP SOC-3640A). Certain fractionization cleanups can be used to selectively remove organochlorine pesticides, aiding in Aroclor determination (CAS SOP SOC-3665). The presence of elemental sulfur will result in interferences for most Aroclors. If GPC cleanup is insufficient, cleanup via Method 3660 (CAS SOP SOC-3660) may be used for the removal of sulfur.

4.3. A standard of the DDT analogs should be injected with each initial calibration to determine which of the PCB or Aroclor peaks may be subject to interferences on the analytical columns used. There may be substantial DDT interference with the last major Aroclor 1254 peak in some soil and sediment samples.

5. SAFETY

5.1. The toxicity or carcinogenicity of each compound or reagent used in this method has not been precisely determined; however, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level.

5.2. Follow all applicable safety procedures as described in the CAS Safety Manual. A reference file of material safety data sheets is available to all personnel involved in these analyses. CAS also maintains a file of OSHA regulations regarding the safe handling of the chemicals specified in this method.

6. SAMPLE COLLECTION, CONTAINERS, PRESERVATION, AND STORAGE

6.1. Containers used to collect samples should be purchased pre-cleaned containers. Alternatively, containers may be soap and water washed followed by methanol (or isopropanol) rinsing. The sample containers should be of glass or teflon and have screw-top covers with teflon liners. In situations where teflon is not available, solvent-rinsed aluminum foil may be used as a liner.

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Highly acidic or basic samples may react with the aluminum foil, causing eventual contamination of the sample. Plastic containers or lids may not be used for the storage of samples due to the possibility of sample contamination from the phthalate esters and other hydrocarbons within the plastic.

6.2. Sample containers should be filled with care so as to prevent any portion of the collected sample coming in contact with the sampler's gloves, thus causing contamination. Samples should not be collected or stored in the presence of exhaust fumes. If the sample comes in contact with the sampler (e.g., if an automatic sampler is used), run reagent water through the sampler and use the rinseate as a field blank.

6.3. Samples should be tested for residual chlorine at the time of sampling. For aqueous samples with residual chlorine present, add 3-mL 10% sodium thiosulfate solution per gallon (0.008%).

6.4. Water and soil samples must be iced or refrigerated at 4 ± 2ºC from time of collection until extraction. Tissue samples should be stored in accordance with project requirements, typically refrigerated or frozen.

6.5. There are no holding time requirements for this method.

7. APPARATUS AND EQUIPMENT

7.1. Gas Chromatograph (GC)

7.1.1. Analytical system complete with gas chromatograph suitable for splitless or on-column automated injection into a wide bore capillary column with an electron capture detector (ECD). Use of Large Volume Injection (LVI) is optional. Helium is used as the carrier gas; argon/methane mixture is used for the detector makeup gas (auxiliary gas). Current instrumental systems are identified as follows:

Instrument I.D.	Analytical System	Routine Matrix
GC09	H-P 5890	Water/Soil/Tissue
GC22	Agilent 6890	Water-LL/Tissue
GC32	Agilent 6890	Water/Soil/Tissue

7.1.2. GC Autosampler: The GC system should be configured with a compatible autosampler for automated injection of standards, samples, and QC samples.

7.1.3. GC Columns - fused silica capillary columns
Column 1: DB-35MS, 30-m x 0.53mm, 1.0um film thickness, or equivalent.
Column 2: DB-XLB, 30-m x 0.53mm, 1.5um film thickness, or equivalent.

Note: Column diameter and film thickness varies depending on the column. Refer to the instrument maintenance logbook for the column used for a specific instrument configuration.

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7.1.4. Data System - A computer data system must be interfaced to the GC/ECD. The system allows the continuous acquisition and storage on machine-readable media of chromatographic data obtained throughout the duration of the analysis program. The computer must have software that includes automated calibration, identification, and quantitation routines. The software must also be capable of integrating the chromatographic peaks abundances. The current version of the manufacturer's software is preferred (Target or HP Chemstation/Enviroquant).

8. STANDARDS, REAGENTS, AND CONSUMABLE MATERIALS

8.1. Solvents: Hexane, acetone, methylene chloride, isooctane, and methanol. Pesticide grade or equivalent.

8.2. Standards

8.2.1. Stock standard solutions may be purchased from a number of vendors. All reference standards, where possible, must be traceable to SI units or NIST certified reference materials.

8.2.2. Aroclor stock standard solutions are purchased from AccuStandard at 1000 ug/mL. Other vendors may be used providing they meet the requirements in sec 8.2.1. Transfer stock standard solutions into Teflon-sealed screw-cap bottles. Stock standard solutions are stored at -10ºC, or at ambient temperature as recommended by the vendor, and protected from light. The expiration date for unopened ampules is the manufacturer's assigned expiration date. If the manufacturer does not assign a date, an expiration date of 1 year from receipt is assigned. Check stock standards frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.

8.2.2.1. Intermediate Aroclor calibration standard solutions are made by diluting 1000 ug/mL stock standards 1:20 in hexane. An intermediate surrogate standard is prepared at 5ug/mL by diluting the stock 1:40 in hexane.

8.2.2.2. Prepare calibration standards at a minimum of five concentration levels containing equal concentrations of both Aroclors 1016 and 1260 by dilution of the intermediate standards with hexane. One of the concentration levels should be at or below a concentration representing the method reporting limit (MRL). The remaining concentration levels should correspond to the expected range of concentrations found in real samples or should define the working range of the GC. See Table 3 for preparation and concentrations, including standards designated as CCVs. A calibration standard of mid-range concentration is used for the CCV.

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8.2.2.3. Calibration standard solutions are stored at 4 ± 2ºC and must be replaced after six months, or sooner, if comparison with check standards indicate a problem.

8.2.3. The independent calibration verification (ICV) standards are prepared purchased from Ultra Scientific at 100 ug/mL. Other vendors may be used providing they meet the requirements in sec 8.2.1. ICV solutions are stored at -10ºC, or at ambient temperature as recommended by the vendor, and protected from light. The expiration date for unopened ampules is the manufacturer's assigned expiration date. If the manufacturer does not assign a date, an expiration date of 1 year from receipt is assigned.

8.2.3.1. Working ICV standards at 1000 ug/mL are prepared as described in Table 3.

8.2.4. Surrogate solutions are prepared from stock solutions purchased from Ultra Scientific at 200 ugmL. Other vendors may be used providing they meet the requirements in sec 8.2.1.

8.2.4.1. The procedure for adding the surrogate solution to the calibration standards is outlined in Table 3.

8.2.4.2. A surrogate spiking solution is prepared at 2 ug/mL by making a 1:100 dilution of the surrogate stock standard in acetone. The surrogate solution is stored in the refrigerator for up to six months.

8.2.5. Standards of the other Aroclors are prepared for use as retention time/pattern standards and to establish calibration factors for these Aroclors (see Table 3).

8.2.6. Matrix spike solution: Prepare a spiking solution at 40 ug/mL containing both Aroclor 1016 and 1260 by diluting the 1000 ug/mL stock standards 1:25 with acetone.

9. PREVENTIVE MAINTENANCE

9.1. All maintenance activities are recorded in a maintenance logbook kept for each instrument. Pertinent information (serial numbers, instrument I.D., etc.) must be in the logbook. This includes the routine maintenance described in section 9. The entry in the log must include: date of event, the initials of who performed the work, and a reference to analytical control.

9.2. Carrier gas - Inline purifiers or scubbers should be in place for all sources of carrier gas. These are selected to remove water, oxygen, and hydrocarbons. Purifiers should be changed as recommended by the supplier.

9.3. Gas Chromatograph

9.3.1. Whenever GC maintenance is performed, care should be taken to minimize the introduction of air or oxygen into the column. Injection port maintenance includes changing the injection port liner, seal, washer, o-ring, septum, column ferrule, and

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autosampler syringe as needed. Liners and seals should be changed when recent sample analyses predict a problem with chomatographic performance. In some cases liners and seals may be cleaned and re-used.

9.3.2. Clipping off a small portion of the head of the column often improves chromatographic performance. When cutting off any portion of the column, make sure the cut is straight and "clean" (uniform, without fragmentation) by using the proper column cutting tool.

9.3.3. Over time, the column will exhibit poorer overall performance, as contaminated sample matrices are analyzed. The length of time for this to occur will depend on the samples analyzed. When a noticeable decrease in column performance is evident and other maintenance options do not result in improvement, the column should be replaced. This is especially true when evident in conjunction with calibration difficulties.

10. RESPONSIBILITIES

10.1. It is the responsibility of the analyst to perform the analysis according to this SOP and to complete all documentation required for data review. Analysis and interpretation of the results are performed by personnel in the laboratory who have demonstrated the ability to generate acceptable results utilizing this SOP. This demonstration is in accordance with the training program of the laboratory. Final review and sign-off of the data is performed by the department supervisor/manager or designee.

10.2. It is the responsibility of the department supervisor/manager to document analyst training. Documenting method proficiency, as described in 8082, is also the responsibility of the department supervisor/manager.

11. PROCEDURE

11.1. Sample Preparation

11.1.1. Water samples (1L) are extracted at a pH of 5-9 with methylene chloride, using Method 3520 (CAS SOP EXT-3520) or using solid phase extraction (CAS SOP EXT-3535). Refer to the applicable extraction SOP. For extraction by 3535, acidification of the sample prior to extraction may be allowable if project objectives and performance requirements of methods 3535 and 8081 are met. An ultra low-level water option may be used, where a 1L sample amount and a final extract volume of 2mL is used. Large Volume Injectors are typically used with this option.

Note: Project-specific or regulation-specific extraction methods may apply. For projects originating from South Carolina and under the SC DHEC lab certification, use the 3520 extraction method only.

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11.1.2. Soil/sediment samples are extracted using either EPA Method 3540 (CAS SOP EXT-3540), EPA Method 3541 (CAS SOP EXT-3541) or EPA Method 3550 (CAS SOP EXT-3550). Refer to the applicable extraction SOP. A low-level sediment option may be used where the sample weight of 40g (20g dry weight) and a final extract volume of 4mL are used.

11.1.3. Additional sample cleanup procedures may be employed as appropriate for the samples. Refer to the section on interferences and the appropriate CAS SOP.

11.2. Calibration

NOTE: The calibration procedure(s) and options chosen must follow the CAS protocols. Any exceptions to the calibration procedures detailed in the CAS SOP for *Calibration of Instruments for Organics Chromatographic Analyses* are described as follows:

Note: Certain state or program protocols have specific procedures for calibration. The analyst must ensure that the correct procedures are used. Known exceptions are as follows:

- The use of quadratic regression calibration is not allowed for projects (samples) originating from South Carolina and under the SC DHEC lab certification.

11.2.1. Prepare a minimum of 5 calibration standards containing equal concentrations of both Aroclor 1016 and 1260 by dilution of the stock standard(s) with isooctane or hexane. Single standards of each of the other target Aroclors are required to aid the analyst in pattern recognition. Once the linearity of the detector has been demonstrated using Aroclor 1016/1260 standards, the single standards of the remaining target Aroclors are also used to determine the calibration factor for each Aroclor. Prepare a standard for each of the other Aroclors. The concentrations should correspond to the mid-point of the linear range of the detector.

Note: DoD QSM requires the quantitation for Aroclors must be performed using a 5-point calibration. Results may not be quantitated using a single point.

A minimum of 3 peaks must be chosen for each Aroclor, and preferably 6 peaks. The peaks must be characteristic of the Aroclor in question. Choose peaks in the Aroclor standards that are at least 25% of the height of the largest Aroclor peak. For each Aroclor, the set of 3 to 6 peaks should include at least one peak that is unique to that Aroclor. Use at least five peaks for the Aroclor 1016/1260 mixture, none of which should be found in both of these Aroclors. Establish the retention time window position using the mid point of the ICAL range before processing the calibration curve.

11.2.2. Calibrate the system immediately prior to conducting any analyses. Refer to Table 2 for instrument conditions. Starting with the standard of lowest concentration, analyze each 1016/1260 calibration standard and tabulate response (peak area) versus the

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concentration in the standard. Refer to Table 2 for instrument conditions. Calculate the ratio of the response to the amount injected the (calibration factor) for each analyte at each standard concentration. For 1016/1260 and DCB, the Relative Standard Deviation (RSD) must be less than 20% when average response factor is used.

11.2.3. Analyze each of the single-point calibration standards of the other target Aroclors. Calculate the calibration factor (CF) for each analyte at each standard concentration.

11.2.4. Each calibration of each Aroclor is verified by an independent source. Prepare an independent calibration verification standard (ICV) by dilution of a stock solution purchased from a different vendor and analyze immediately after each initial calibration. Calculate the concentration using the typical procedure used for quantitation. Calculate the percent difference (%D) from the ICV true value. Evaluate the ICV as described in the SOP for *Calibration of Instruments for Organics Chromatographic Analyses.*

11.3. Calibration Verification

11.3.1. The working calibration curve or calibration factor must be verified on each analytical sequence by the analysis of one or more mid-range calibration standards (CCV). A standard (CCV) must be injected at the start of each sequence and after each set of sample extracts (every 10 samples or every 12 hours, whichever is first) in the analysis sequence.

Note: DoD projects require a CCV analysis every 10 field samples.

11.4. Retention Time Windows

11.4.1. Pattern recognition/matching and retention times are used for the identification of PCBs as Aroclors.

11.4.2. Establish retention time windows for the peaks used for quantitation with the GC system in acceptable operating condition. Make three injections of all analytes throughout the course of a 72-hour period. Serial injections over less than a 72-hour period may result in retention time windows that are too tight. Using retention times from these analyses, calculate retention time windows. Refer to EPA Method 8000C for detailed instructions.

11.4.3. Plus or minus three times the standard deviation of the absolute retention times for each standard will be used to define the retention time window; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms. In those cases where the standard deviation for a particular standard is zero, the laboratory may use a default window of ± 0.03 minutes. If the peak width is > 0.06 minutes, use a default window of 0.1 minutes.

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11.4.4. Calculate retention time windows for each standard on each GC column and whenever a new GC column is installed. Retain this data in the method file.

11.5. Gas Chromatography

11.5.1. Set up an analytical sequence for the standards and samples to be analyzed. Calibrate the system as described in Section 11.2. Refer to Table 2 for typical instrument operating conditions. The same conditions must be used for samples as for calibration and QC analyses. Ensure that the instrument configuration is correct and that any necessary maintenance has been performed. Figure 1 shows a typical analysis sequence.

Note: For DoD projects, the CCB must be analyzed following the CCV. Instrument blanks/CCBs may not be analyzed prior to QC samples or standards.

11.5.2. Evaluate the CCVs as indicated in Section 11.3. Use the standards interspersed throughout the sample analysis sequence to evaluate the qualitative performance of the GC system including positioning of the retention time window. If any retention time shift which would impede analyte identification is evident (as shown by Aroclor pattern irregularities or the surrogate falling outside of the retention time window), evaluate the chromatogram for possible causes such as carryover from a highly contaminated sample. If a problem related to GC system has been determined to be the cause of retention time shift, perform whatever maintenance is necessary before reanalyzing the CCV or recalibrating and proceeding with sample analysis. All samples that were injected after the sample exceeded the criteria must be reinjected if initial analysis indicated the presence of any analytes of interest.

FIGURE 1

Analysis Sequence

Initial Calibration Blank	ICB
Standard 1	1221 midpoint
Standard 2	1232 midpoint
Standard 3	1242 midpoint
Standard 4	1248 midpoint
Standard 5	1254 midpoint
Standard 6	1262 midpoint
Standard 7	1268 midpoint
Standards 8-12	1016/1260 ICAL standards
Standards 13-20	ICVs for 1016, 1221, 1232, 1242, 1254, 1260, 1262, 1268
Continuing Calibration Verification	CCV
Method Blank	
Laboratory Control Sample	LCS

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Sample 1 - 8	
Matrix Spike	
Duplicate Matrix Spike	
Continuing Calibration Verification	CCV
Continuing Calibration Blank	CCB
Sample 9 - 18	
Continuing Calibration Verification	CCV
Continuing Calibration Blank	CCB

Note: For DoD projects, the CCB must be analyzed following the CCV. Instrument blanks and CCBs may not be analyzed prior to QC samples or standards.

11.6. Troubleshooting

11.6.1. Initial calibration – If the initial calibration fails to meet the criteria, or the ICV fails (indicating a calibration problem), the following steps may be taken, depending on nature of the problem.

- Recheck the information entered into the software used for calibration and quantitation. Verify the standard values are correct and datafiles are correct. If incorrect, repeat the calibration with the correct information.
- Recheck standards preparation to ensure that standards are correct. Re-prepare and reanalyze if needed.
- Ensure that proper preventive maintenance was performed. Repeat the preventive maintenance if necessary and reanalyze the calibration.
- If calibration problems persist or more substantial calibration problems exist, corrective maintenance or repair may be needed. This includes such measures as column changes, detector maintenance, or GC repair. This will depend on the nature of the problem. Following any such maintenance, repeat the calibration.

11.6.2. Continuing calibration – If the CCV analysis fails to meet the criteria, the following steps may be taken, depending on nature of the problem.

- Recheck the information entered into the software used for calibration and quantitation. Verify the standard values are correct and datafiles are correct. If incorrect, repeat the calibration with the correct information.
- Recheck standards preparation to ensure that standards are correct and that the correct standard is used as the CCV. Re-prepare and reanalyze if needed. Note that NELAC and DoD requirements apply when multiple CCVs are analyzed.
- Ensure that proper preventive maintenance was performed. Repeat the preventive maintenance if necessary and reanalyze the CCV.

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- If calibration problems persist or more substantial calibration problems exist, corrective maintenance or repair may be needed. This includes such measures as column changes, detector maintenance, or GC repair. This will depend on the nature of the problem. Following any such maintenance, repeat analysis of the CCV and necessary samples. Major maintenance will require recalibration. Note that some samples may quickly deteriorate the system to the point that closing CCVs will not pass. This should be verified through a second run of the samples and documented.

12. QA/QC REQUIREMENTS

12.1. Initial Precision and Recovery Validation

12.1.1. The accuracy and precision of the procedure must be validated before analyses of samples begin, or whenever significant changes to the procedures have been made. To do this, four water samples are spiked with the LCS spike solution, then prepared and analyzed.

12.2. Method Detection Limits and Method Reporting Limits

12.2.1. A method detection limit (MDL) study must be undertaken before analysis of samples can begin. To establish detection limits that are precise and accurate, the analyst must perform the following procedure. Spike seven blank matrix (water or soil) samples with MDL spiking solution at a level below the MRL. Follow the analysis procedures in Section 11 to analyze the samples.

12.2.2. Calculate the average concentration found (x) in µg/mL, and the standard deviation of the concentrations (s) in µg/mL for each analyte. Calculate the MDL for each analyte. Refer to the CAS *SOP Performing Method Detection Limit Studies and Establishing Limits of detection and Quantification (ADM-MDL).* The MDL study must be verified annually.

12.2.3. Limits of Quantification (LOQ)

12.2.3.1. The laboratory establishes a LOQ for each analyte as the lowest reliable laboratory reporting concentration or in most cases the lowest point in the calibration curve which is less than or equal to the desired regulatory action levels, based on the stated project requirements. Analysis at the lowest point calibration level provides confirmation of the established sensitivity of the method. The LOQ recoveries should be within 50% of the true values to verify the data reporting limit. Refer to the CAS SOP *Performing Method Detection Limit Studies and Establishing Limits of detection and Quantification (ADM-MDL).*

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12.2.4. The Method Reporting Limits (MRLs) used at CAS are the routinely reported lower limits of quantitation which take into account day-to-day fluctuations in instrument sensitivity as well as other factors. These MRLs are the levels to which CAS routinely reports results in order to minimize false positive or false negative results. The MRL is normally two to ten times the method detection limit.

12.3. Ongoing QC Samples required are described in the CAS-Kelso Quality Assurance Manual and in the SOP for Sample Batches. Additional QC Samples may be required in project specific quality assurance plans (QAPP). For example projects managed under the DoD ELAP must follow requirements defined in the DoD *Quality Systems Manual for Environmental Laboratories.* General QA requirements for DoD QSM are defined in the laboratory SOP, Department *of Defense Projects – Laboratory Practices and Project Management (ADM-DOD).* General QC Samples are:

12.3.1. Method blank - A method blank is extracted and analyzed with every batch of 20 or fewer samples to demonstrate that there are no method interferences. The method blank must demonstrate that interferences from the analytical and preparation steps minimized. No target analytes should be detected above the MRL in the method blank.

12.3.1.1. If the method blank fails to meet the criteria, the sample data in the associated batch should be examined. If all samples and QC have hits for the analyte, samples and QC should be re-extracted and reanalyzed as necessary (samples with higher level hits may not need reanalysis). It should be verified through the analysis of instrument blanks that the problem is isolated to either the GC of the sample preparation. If the problem is isolated to the MB, the data may be flagged and narrated. Also refer to the QA Manual for additional corrective action.

12.3.1.2. The source of MB contamination should be isolated and corrected as soon as possible to prevent further failures.

Note: DoD projects require no analytes detected > ½ the RL or > 1/10 the regulatory limit.

12.3.2. A lab control sample (LCS) must be extracted and analyzed with every batch of 20 samples. The water LCS is prepared by adding 50 µL of the matrix spike solution to 1L of reagent water, resulting in a concentration of 2.0 ug/L. The soil LCS is prepared by adding 100 µL of spike solution to 20g of sand, resulting in a concentration of 200 ug/kg. For project-specific low-level extractions, spiking amounts can be adjusted accordingly. Calculate percent recovery (%R) as follows:

$$\%R = X/TV \times 100$$

Where X = Concentration of the analyte recovered
TV = True value of amount spiked

Acceptance criteria for lab control samples are listed in Table 1.

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12.3.3. Project-specific or program-specific acceptance criteria may supersede CAS criteria. For example, for samples requiring South Carolina DHEC certification the acceptance criteria are 70-130 % recovery. If the lab control sample (LCS) fails acceptance limits for any of the compounds, the analyst must evaluate the system and calibration. If no problems are found, corrective action must be taken. The acceptance criteria listed are current criteria, but are subject to change as control limits are updated.

12.3.4. A matrix spike/duplicate matrix spike (MS/DMS) must be extracted and analyzed with every batch of 20 or fewer samples. The MS/DMS is prepared by adding the same volume of the matrix spike solution to the sample as listed for the LCS, then proceeding with the entire extraction and analysis. Calculate percent recovery (%R) as follows:

$$\%R = \frac{X - X1}{TV} \times 100$$

Where X = Concentration of the analyte recovered
X1 = Concentration of unspiked analyte
TV = True value of amount spiked

Calculate Relative Percent Difference (RPD) as:

$$RPD = \frac{|R1 - R2|}{(R1 + R2)/2} \times 100$$

Where R1 = % recovery of the MS
R2 = % recovery of the DMS

Acceptance criteria for matrix spikes are listed in Table 1. If the MS/DMS recovery is out of acceptance limits for reasons other than matrix effects, corrective action must be taken. The acceptance criteria listed are current criteria, but are subject to change as control limits are updated.

12.3.5. Surrogate spike is added to every sample, blank and spike prior to extraction. Two surrogate standards (tetrachloro-m-xylene and decachlorobiphenyl) are added to each sample. For water, 100µL of the surrogate spike is added to 1L, resulting in 0.2 ug/L. For soil, 200µL of the surrogate spike is added to 20g, resulting in 20 ug/kg. Calculate surrogate percent recovery (%R) as:

%R = S/V x 100
Where S = The amount of surrogate recovered
V = The amount spiked/final volume

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The acceptance limits for the surrogates are given in Table 1. Both surrogate recoveries must be within the acceptance limits. If either (or both) surrogate is outside of acceptance limits for reasons other than matrix interferences, corrective action must be taken. Corrective actions include recalculation, reanalysis, or reextraction and reanalysis. The acceptance criteria listed are current criteria, but are subject to change as control limits are updated.

12.3.6. Control charts should be maintained for QC results. The charts should be reviewed periodically for trends in results. Control limits for QC analyses may be determined using the control charts or similar mechanism on an annual basis.

13. DATA REDUCTION, REVIEW, AND REPORTING

13.1. Identification of PCBs as Aroclors

13.1.1. To identify Aroclors, compare the chromatographic pattern of the sample to known Aroclor standards. Tentative identification of PCBs as Aroclors is made when the pattern of peaks in the sample chromatogram matches the pattern of peaks in the Aroclor standard itself. There also needs to be agreement between the retention times and response ratios of the 3-6 selected quantitation peaks in the sample chromatogram and the Aroclor standard.

13.1.2. Tentative identification of analytes must be confirmed using a second GC column of dissimilar phase. Identify the Aroclor by comparing the chromatographic pattern of the sample to known Aroclor standards analyzed on the same column. Confirmation of the Aroclor is made when the sample chromatogram matches the pattern of peaks in the Aroclor being confirmed. Quantitations for the 2 columns must agree ($\leq$ 40%RPD) to confirm the identification. If interferences or other sample anomalies make the RPD value >40% but the analyst makes a positive identification, the basis of the identification must be documented and the data user notified of the discrepancy (see section 13.2).

13.2. Sample matrix difficulties

13.2.1. Weathering of PCBs in the environment and changes resulting from waste treatment processes may alter the pattern of a specific Aroclor so it does not closely match an Aroclor standard. The earlier eluting peaks will often diminish in comparison to the later eluting peaks. If this is observed, alternate peaks may be selected to aid identification to reduce quantitation bias.

13.2.2. Metabolism by organisms may also alter the pattern since individual PCB congeners are metabolized at different rates. When working with tissue samples, the 40% RPD criteria for confirmation may not be met.

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13.2.3. Samples may also include mixtures of two or more Aroclors. To the extent possible, identify and quantify each Aroclor.

13.2.4. High amounts of organochlorine pesticides in the sample may interfere with identification. If this is observed, alternate peaks may be selected to aid identification to reduce quantitation bias. Certain fractionization cleanups can be used to selectively remove organochlorine pesticides, aiding in Aroclor determination (Acid cleanup, CAS SOP SOC-3665).

13.2.5. For all of these reasons a high level of analyst expertise is required to interpret complex chromatograms.

13.3. Quantitation of PCBs as Aroclors:

13.3.1. The quantitation of PCBs as Aroclors is accomplished by comparison of the sample chromatogram to that of the most similar Aroclor standard or standards. All calibration acceptance criteria as described in section 11must be met before reporting any results. Sample results should then be reported according to the organics confirmation SOP (SOC-CONF). Results may be reported from either column if all calibration acceptance criteria as described in section 11are met.

13.3.2. Once the Aroclor pattern has been identified, compare the responses of 3 to 6 major peaks in the calibration standard of that Aroclor with the peaks observed in the sample extract. The amount of Aroclor is calculated using the individual calibration factor for each of the 3 to 6 peaks and the calibration model selected in section 11. The concentration is determined using the 3 to 6 characteristic peaks and then the concentrations are averaged to determine the concentration of the Aroclor. If there are interfering peaks with the 3 to 6 quantitation peaks that cause Aroclor average to be falsely overstated, then that interference peak is Q-deleted using the data system and the average is recalculated so that the average more truly represents the concentration in the sample. This often occurs when there are more than one Aroclor in a sample extract or if pesticides are present. Quantitation of mixed Aroclors will require the selection of peaks that are not shared in common by both Aroclors.

13.3.3. For samples with severe matrix interferences, the quantitation may be performed by measuring the total area of the PCB pattern and quantifying on the basis of the Aroclor standard that is most similar to the sample. Any peaks that are not identifiable as PCBs should be subtracted from the total area. When the quantitation option is used, the sample problems should be described for the data user and quantification procedure documented.

13.3.4. Using the data system, calculate the concentration in the extract using the calibration model chosen for calibration (see the SOP SOC-CAL).

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13.3.5. Using the data system, calculate the concentration of each analyte in the sample extract (Cex) µg/ml units using the calibration factor or calibration curve (Section 11). The sample concentration computed using the following equations:

Aqueous Samples:

$$Concentration\ (\mu g / L) = \frac{(Cex)\ (Vf)\ (D)}{(Vs)}$$

Where Cex = Concentration in extract in µg/ml
Vf = Final volume of extract in ml
D = Dilution factor
Vs = Volume of sample extracted, liters

Nonaqueous Samples:

$$Concentration\ (mg / Kg) = \frac{(Cex)\ (Vf)\ (D)\ x\ 1{,}000}{(W)\ x\ 1{,}000}$$

Where Cex = Concentration in extract in µg/ml
Vf = Final volume of extract in ml
D = Dilution factor
W = Weight of sample extracted. The wet or dry weight may be used, depending upon the specific client requirements.

13.4. Sample concentrations are reported when all QC criteria for the analysis have been met or the results are qualified with a footnote.

13.5. Data Review

13.5.1. Following primary data interpretation and calculations, all data is reviewed by a secondary analyst. Following generation of the report, the report is also reviewed. Refer to the *SOP for Laboratory Data Review Process* for details. The person responsible for final review of the data report and/or data package should assess the overall validity and quality of the results and provide any appropriate comments and information to the Project Chemist to inclusion in the report narrative.

13.6. Reporting

13.6.1. Reports are generated using the STEALTH Data Reporting System which compiles the SMO login information. This compilation is then transferred to a file, which

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STEALTH uses to generate a report. The forms generated may be CAS standard reports, DOD, or client-specific reports. The compiled data from LIMS is also used to create EDDs.

13.6.2. As an alternative, reports are generated using Excel© templates located in R:\SVG\forms. The analyst should choose the appropriate form and QC pages to correspond to required tier level and deliverables requirements. The detected analytes, surrogate and matrix spikes are then transferred, by hand or electronically, to the templates.

13.6.3. Sample concentrations are reported when all QC criteria for the analysis have been met or the results are qualified with an appropriate footnote. For Arizona projects the appropriate Arizona qualifier must be used.

14. CORRECTIVE ACTION

14.1. Refer to the SOP for *Corrective Action* for procedures for corrective action. Personnel at all levels and positions in the laboratory are to be alert to identifying problems and nonconformities when errors, deficiencies, or out-of-control situations are detected.

14.2. Handling out-of-control or unacceptable data

14.2.1. On-the-spot corrective actions that are routinely made by analysts and result in acceptable analyses should be documented as normal operating procedures, and no specific documentation need be made other than notations in laboratory maintenance logbooks, runlogs, for example.

14.2.2. Documentation of a nonconformity must be done using a Nonconformity and Corrective Action Report (NCAR) when:

- Corrective action is not taken or not possible
- Corrective action fails to correct an out-of-control problem on a laboratory QC or calibration analysis.
- Reanalysis corrects the nonconformity but is not a procedurally compliant analysis.

15. METHOD PERFORMANCE

15.1. This method was validated through single laboratory studies of accuracy and precision. Refer to the reference method for additional method performance data available.

15.2. The method detection limit (MDL) is established using the procedure described in the SOP for The Determination of Method Detection Limits (ADM-MDL). Method Reporting Limits are established for this method based on MDL studies and as specified in the CAS Quality Assurance Manual.

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16. TRAINING

16.1. Training outline

16.1.1. Review literature (see references section). Read and understand the SOP. Also review the applicable MSDS for all reagents and standards used. Following these reviews, observe the procedure as performed by an experienced analyst at least three times.

16.1.2. The next training step is to assist in the procedure under the guidance of an experienced analyst for a period of 3 months. During this period, the analyst is expected to transition from a role of assisting, to performing the procedure with minimal oversight from an experienced analyst.

16.1.3. Perform initial precision and recovery (IPR) study as described above for water samples. Summaries of the IPR are reviewed and signed by the supervisor. Copies may be forwarded to the employee's training file. For applicable tests, IPR studies should be performed in order to be equivalent to NELAC's Initial Demonstration of Capability.

16.2. Training is documented following the SOP for Documentation of Technical Personnel Training.

16.2.1. NOTE: When the analyst training is documented by the supervisor on internal training documentation forms, the supervisor is acknowledging that the analyst has read and understands this SOP and that adequate training has been given to the analyst to competently perform the analysis independently.

17. POLLUTION PREVENTION

It is the laboratory's practice to minimize the amount of solvents and reagents used to perform this method wherever technically sound, feasibly possible, and within method requirements. Standards are prepared in volumes consistent with laboratory use in order to minimize the volume of expired standards to be disposed of. The threat to the environment from solvents and/or reagents used in this method may be minimized when recycled or disposed of properly.

18. WASTE MANAGEMENT

The laboratory will comply with all Federal, State, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions as specified in the laboratory Safety Manual, Sections 6 and 7.

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19. METHOD MODIFICATIONS

19.1. There are no known modifications in this laboratory standard operating procedure from the reference method.

20. REFERENCES

18.1. Polychlorinated Biphenyls (PCBs) as Aroclors, Method 8082A, Revision 1, February 2007, EPA Test Methods for Evaluating Solid Waste, SW-846, Update IV

18.2. Determinative Chromatographic Separations, EPA SW846, Test Methods For Evaluating Solid Waste, On-Line, Method 8000C, Revision 3, March 2003.

18.3. 8000C Method criteria, Arizona DHS, 2/13/2007. Available online at http://www.azdhs.gov/lab/license/tech/8000cmethod.pdf

18.4. *DoD Quality Systems Manual for Environmental Laboratories* Version 4.2 10/25/10.

21. CHANGES SINCE THE LAST REVISION

21.1. Sec 1 removed PQL – definition changed
21.2. Sec 3 – added definition for LCS
21.3. Sec 4.3 removed the words ‘and technical Chlordane’
21.4. Sec 6.5 – removed HT requirements to comply with SW-846 table 4-1
21.5. Sec 8 completely re-written and re-organized.for clarity
21.6. Sec 8.2.1 is new
21.7. Sec 8.2.2 removed first sentence that was in previous revision
21.8. Sec 9.1 re-written to comply with current accreditation standards
21.9. Sec 11.5.2 - Figure 1 was edited to change the order of CCB and CCV information. Remove first Note that was in previous revision – did not make sense.
21.10. Sec 13.3.1 corrected typos
21.11. Sec 19 is new
21.12. Sec 20 – added 8000C reference

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CAS/KELSO DATA QUALITY OBJECTIVES – Table 1

METHOD	ANALYTE	CAS No.	MATRIX	MDLa	MRL	LODb	LOQc	UNITS	Accuracy (LCS %Rec.)	Matrix Spike (%Rec.)	Precision (% RPD)
8082	Aroclor 1016	12674-11-2	Soil	0.019	0.10	0.038	0.10	mg/kg	40-128	40-128	40
8082	Aroclor 1221	11104-28-2	Soil	0.019	0.20	0.038	0.20	mg/kg	-	-	-
8082	Aroclor 1232	11141-16-5	Soil	0.019	0.10	0.038	0.10	mg/kg	-	-	-
8082	Aroclor 1242	53469-21-9	Soil	0.019	0.10	0.038	0.10	mg/kg	-	-	-
8082	Aroclor 1248	12672-29-6	Soil	0.019	0.10	0.038	0.10	mg/kg	-	-	-
8082	Aroclor 1254	11097-69-1	Soil	0.019	0.10	0.038	0.10	mg/kg	-	-	-
8082	Aroclor 1260	11096-82-5	Soil	0.019	0.10	0.038	0.10	mg/kg	42-122	42-122	40
8082	Aroclor 1262	37324-23-5	Soil	0.019	0.10	0.038	0.10	mg/kg	-	-	-
8082	Aroclor 1268	11100-14-4	Soil	0.019	0.10	0.038	0.10	mg/kg	-	-	-
8082	Aroclor 5432	63496-31-1	Soil	2.1	20	6.3	20	ug/Kg	70-130	70-130	40
8082	Aroclor 5442	12642-23-8	Soil	2.1	20	6.3	20	ug/Kg	70-130	70-130	40
8082	Aroclor 5460	11126-42-4	Soil	2.1	100	6.3	100	ug/Kg	70-130	70-130	40
8082	Decachlorobiphenyl (Surr.)	2051-24-3	Soil	NA	NA	NA	NA	%	35-133	NA	NA
8082	Tetrachloro-m-xylene (Surr.)	877-09-8	Soil	NA	NA	NA	NA	%	30-120	NA	NA
8082	Aroclor 1016	12674-11-2	Water	0.049	0.2	0.1	0.2	ug/L	50-115	50-115	30
8082	Aroclor 1221	11104-28-2	Water	0.049	0.4	0.2	0.4	ug/L	-	-	-
8082	Aroclor 1232	11141-16-5	Water	0.049	0.2	0.1	0.2	ug/L	-	-	-
8082	Aroclor 1242	53469-21-9	Water	0.049	0.2	0.1	0.2	ug/L	-	-	-
8082	Aroclor 1248	12672-29-6	Water	0.049	0.2	0.1	0.2	ug/L	-	-	-
8082	Aroclor 1254	11097-69-1	Water	0.049	0.2	0.1	0.2	ug/L	-	-	-
8082	Aroclor 1260	11096-82-5	Water	0.049	0.2	0.1	0.2	ug/L	50-115	50-115	30
8082	Aroclor 1262	37324-23-5	Water	0.049	0.2	0.1	0.2	ug/L	-	-	-
8082	Aroclor 1268	11100-14-4	Water	0.049	0.2	0.1	0.2	ug/L	-	-	-
8082	Decachlorobiphenyl (Surr.)	2051-24-3	Water	NA	NA	NA	NA	%	39-128	NA	NA
8082	Tetrachloro-m-xylene (Surr.)	877-09-8	Water	NA	NA	NA	NA	%	31-94	NA	NA

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CAS/KELSO DATA QUALITY OBJECTIVES – Table 1

8082	Aroclor 1016	12674-11-2	Tissue	2.4	10			ug/Kg	46-128	46-128	40
8082	Aroclor 1221	11104-28-2	Tissue	2.4	20			ug/Kg	-	-	-
8082	Aroclor 1232	11141-16-5	Tissue	2.4	10			ug/Kg	-	-	-
8082	Aroclor 1242	53469-21-9	Tissue	2.4	10			ug/Kg	-	-	-
8082	Aroclor 1248	12672-29-6	Tissue	2.4	10			ug/Kg	-	-	-
8082	Aroclor 1254	11097-69-1	Tissue	2.4	10			ug/Kg	-	-	-
8082	Aroclor 1260	11096-82-5	Tissue	2.4	10			ug/Kg	46-128	46-128	40
8082	Aroclor 1262	37324-23-5	Tissue	2.4	10			ug/Kg	-	-	-
8082	Aroclor 1268	11100-14-4	Tissue	2.4	10			ug/Kg	-	-	-
8082	Decachlorobiphenyl (Surr.)	2051-24-3	Tissue	NA	NA	NA	NA	%	37-139	NA	NA
8082	Tetrachloro-m-xylene (Surr.)	877-09-8	Tissue	NA	NA	NA	NA	%	33-120	NA	NA

a Method Detection Limits are subject to change as new MDL studies are completed.

a MDL is the smallest analyte concentration that can be demonstrated to be different from zero with 99% confidence

b The LOD is the smallest amount of a substance that must be present in a sample in order to be detected with 99% confidence.

Verification is acceptable if the response is > 3x instrument noise & 2nd column confirmed or pattern.

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CAS/KELSO DATA QUALITY OBJECTIVES – Table 1

METHOD	ANALYTE	CAS No.	MATRIX	MDLa	MRL	LODb	LOQc	UNITS	Accuracy (LCS %Rec.)	Matrix Spike (%Rec.)	Precision (% RPD)
8082 LL	Aroclor 1016	12674-11-2	Soil	2.1	10	4.25	10	ug/kg	40-128	40-128	40
8082 LL	Aroclor 1221	11104-28-2	Soil	2.1	20	4.25	20	ug/kg	-	-	-
8082 LL	Aroclor 1232	11141-16-5	Soil	2.1	10	4.25	10	ug/kg	-	-	-
8082 LL	Aroclor 1242	53469-21-9	Soil	2.1	10	4.25	10	ug/kg	-	-	-
8082 LL	Aroclor 1248	12672-29-6	Soil	2.1	10	4.25	10	ug/kg	-	-	-
8082 LL	Aroclor 1254	11097-69-1	Soil	2.1	10	4.25	10	ug/kg	-	-	-
8082 LL	Aroclor 1260	11096-82-5	Soil	2.1	10	4.25	10	ug/kg	42-122	42-122	40
8082 LL	Aroclor 1262	37324-23-5	Soil	2.1	10	4.25	10	ug/kg	-	-	-
8082 LL	Aroclor 1268	11100-14-4	Soil	2.1	10	4.25	10	ug/kg	-	-	-
8082 LL	Aroclor 1016	12674-11-2	Water	0.00096	0.005	0.002	0.006	ug/L	50-115	50-115	30
8082 LL	Aroclor 1221	11104-28-2	Water	0.00096	0.010	0.01	0.025	ug/L	-	-	-
8082 LL	Aroclor 1232	11141-16-5	Water	0.00096	0.005	0.002	0.006	ug/L	-	-	-
8082 LL	Aroclor 1242	53469-21-9	Water	0.00096	0.005	0.002	0.006	ug/L	-	-	-
8082 LL	Aroclor 1248	12672-29-6	Water	0.00096	0.005	0.002	0.006	ug/L	-	-	-
8082 LL	Aroclor 1254	11097-69-1	Water	0.00096	0.005	0.002	0.006	ug/L	-	-	-
8082 LL	Aroclor 1260	11096-82-5	Water	0.00096	0.005	0.002	0.006	ug/L	50-115	50-115	30
8082 LL	Aroclor 1262	37324-23-5	Water	0.00096	0.005	0.002	0.006	ug/L	-	-	-
8082 LL	Aroclor 1268	11100-14-4	Water	0.00096	0.005	0.002	0.006	ug/L	-	-	-

a Method Detection Limits are subject to change as new MDL studies are completed.

a MDL is the smallest analyte concentration that can be demonstrated to be different from zero with 99% confidence

b The LOD is the smallest amount of a substance that must be present in a sample in order to be detected with 99% confidence.

Verification is acceptable if the response is > 3x instrument noise & 2nd column confirmed or pattern.

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TABLE 2

Gas Chromatograph Operating Conditions*

Gas Chromatograph:	Hewlett-Packard Model 5890 or equivalent w/ECD
Injection Port Temperature:	300C
Oven Temperature Program:	160C for 0.5 min., 30º/min ramp to 210C, then 10º/min. to 300C, hold 11.03 min.
Detector Temperature:	350C
Injection Volume:	1 µL
Column:	30 m, DB-35MS and 30 m DB-XLB*
Carrier Gas:	Helium
Auxillary Gas:	Argon/Methane
Data System:	HP Chemstation (acquisition) and Target (data)

* The instrument temperatures may be modified depending on the instrument used. Also, the GC column diameter and film thickness depend on the instrument used. All conditions must be the same for initial calibration, continuing calibration, sample, and QC analyses.

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TABLE 3

CALIBRATION STANDARD PREPARATION

1016/1260 Initial Calibration Standards (prepared in hexane)

Aroclor 1016 50 ug/mL Intermediate	Aroclor 1260 50 ug/mL Intermediate	Surrogate 5 ug/mL Intermediate	Final Volume	Final Concentration Aroclors	Final Concentration Surrogates
12.5 uL	12.5 uL	12.5 uL	25 mL	25 ug/L	2.5 ug/L
25 uL	25 uL	25 uL	25 mL	50 ug/L	5.0 ug/L
250 uL	250 uL	250 uL	25 mL	500 ug/L	50 ug/L
500 uL	500 uL	500 uL	25 mL	1000 ug/L*	100 ug/L*
1000 uL	1000 uL	1000 uL	25 mL	2000 ug/L	200 ug/L
2500 uL	2500 uL	2500 uL	25 mL	5000 ug/L	500 ug/L

* CCV Standard

Single-Point Calibration Standards

Intermediate Standard (1000 ug/mL)	Aliquot	Final Volume	Solvent	Final Concentration
Aroclor 1221	25 uL	25 mL	Hexane	1000 ug/L
Aroclor 1232	25 uL	25 mL	↓	1000 ug/L
Aroclor 1242	25 uL	25 mL	↓	1000 ug/L
Aroclor 1248	25 uL	25 mL	↓	1000 ug/L
Aroclor 1254	25 uL	25 mL	↓	1000 ug/L
Aroclor 1262	25 uL	25 mL	↓	1000 ug/L
Aroclor 1268	25 uL	25 mL	↓	1000 ug/L

ICV Standards

ICV Stock Standard (100 ug/mL)	Aliquot	Final Volume	Solvent	Final Concentration
Aroclor 1016	250 uL	25 mL	Hexane	1000 ug/L
Aroclor 1221	250 uL	25 mL	↓	1000 ug/L
Aroclor 1232	250 uL	25 mL	↓	1000 ug/L
Aroclor 1242	250 uL	25 mL	↓	1000 ug/L
Aroclor 1248	250 uL	25 mL	↓	1000 ug/L
Aroclor 1254	250 uL	25 mL	↓	1000 ug/L
Aroclor 1260	250 uL	25 mL	↓	1000 ug/L
Aroclor 1262	250 uL	25 mL	↓	1000 ug/L
Aroclor 1268	250 uL	25 mL	↓	1000 ug/L

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STANDARD OPERATING PROCEDURE

CONGENER-SPECIFIC DETERMINATION OF POLYCHLORINATED BIPHENYLS (PCBs) BY GAS CHROMATOGRAPHY/ELECTRON CAPTURE DETECTION (GC/ECD)

SOC-8082Co
Revision 12

Effective Date: November 5, 2011

Approved By:

Supervisor	10/6/11 Date
QA Manager	10/6/11 Date
Laboratory Director	10/6/11 Date

COLUMBIA ANALYTICAL SERVICES, INC.
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CONGENER-SPECIFIC DETERMINATION OF POLYCHLORINATED BIPHENYLS (PCBS) BY GAS CHROMATOGRAPHY/ELECTRON CAPTURE DETECTION (GC/ECD)

1. SCOPE AND APPLICATION

1.1. This procedure is used specifically to determine the concentrations of certain PCB congeners in water, soil and sediment using EPA method 8082A. This procedure may also be applicable to various tissue samples. The procedure is intended to determine these compounds at trace levels and therefore is not applicable to miscellaneous waste matrices and oils. More appropriate methods are available for the determination of PCBs (as Aroclors) in waste matrices.

1.2. There are 209 identified congeners for which the method may possibly be applied. Table 1 lists the analytes that can routinely be determined by this procedure and lists the method reporting limits (MRLs) for each compound in water and soil. Equivalent nomenclature for MRL includes Estimated Quantitation Limit (EQL). Therefore, MRL=EQL. The reported MRL may be adjusted if required for specific project requirements; however, the capability of achieving other reported MRLs must be demonstrated. The Method Detection Limits (MDLs) that have been achieved are given in Table 2 and are subject to change as MDL studies are updated.

1.3. In cases where there is a project-specific quality assurance plan (QAPP), the project manager identifies and communicates the QAPP-specific requirements to the laboratory. In general, project specific QAPP's supersede method specified requirements. An example of this are projects falling under DoD ELAP. QC requirements defined in the SOP *Department of Defense Projects – Laboratory Practices and Project Management (ADM-DOD)* may supersede the requirements defined in this SOP.

2. METHOD SUMMARY

This procedure is based on EPA Method 8082A. This procedure provides sample preparation techniques and GC/ECD conditions for the detection of PCB congeners at trace levels. Samples are extracted using an appropriate technique for the sample matrix. Cleanup steps are used to remove unwanted co-extracted compounds and/or matrix interferences. Depending on the sample matrix, some cleanup steps may not be necessary. The final sample extract is analyzed by simultaneous dual column gas chromatography using an electron capture detector. The compounds are separated on fused silica capillary columns with differing chromatographic separation characteristics. Identification of the analytes of interest is performed by comparing the retention times of the analytes with the respective retention times of an authentic standard. Quantitative analysis is performed by using the authentic standard to produce a response factor or calibration curve. The calibration data is used to determine the concentration of an analyte in the extract. The concentration in the sample is calculated using the sample weight or volume and the extract volume.

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3. DEFINITIONS

Analysis Sequence - Samples are analyzed in a set referred to as an analysis sequence. The sequence begins with instrument calibration followed by sample extracts interspersed with calibration standards. The sequence ends when the set of samples has been injected or when qualitative and/or quantitative QC criteria are exceeded.

Initial (or Independent) Calibration Verification (ICV) - Initial calibration verification standards which are analyzed after initial calibration with newly prepared standards but prior to sample analysis, in order to verify the validity of the standards used in calibration. The ICV standards are prepared from materials obtained from a source different from that used to prepare calibration standards.

Matrix Spike/Duplicate Matrix Spike Analysis (MS/DMS) - In the matrix spike analysis, predetermined quantities of stock solutions of selected PCB congeners are added to a sample matrix prior to sample extraction and analysis. The purpose of the matrix spike is to evaluate the effects of the sample matrix on the methods used for the analyses. Samples are split into duplicates, spiked, and analyzed. Percent recoveries are calculated for each of the analytes detected. The relative percent difference between the samples is calculated and used to assess analytical precision. The concentration of the spike should be at 5 to 10 times the MRL or at levels specified by a project analysis plan.

Standard Curve - A standard curve is a calibration curve which plots concentrations of a known analyte standard versus the instrument response to the analyte.

Surrogate - Surrogates are organic compounds which are similar to analytes of interest in chemical composition, extraction, and chromatography, but which are not normally found in environmental samples. The purpose of the surrogates is to evaluate the preparation and analysis of samples. These compounds are spiked into all blanks, standards, samples, and spiked samples prior to analysis. Percent recoveries are calculated for each surrogate.

Method Blank - The method blank is an artificial sample designed to monitor introduction of artifacts into the process. The method blank is carried through the entire analytical procedure.

Continuing Calibration Verification Standard (CCV) - A mid-level standard injected into the instrument at specified intervals and is used to verify the initial calibration.

Instrument Blank (CCB) - The instrument blank (also called continuing calibration blank) is a volume of clean solvent analyzed on each GC column and instrument used for sample analysis. The purpose of the instrument blank is to determine the levels of contamination associated with the instrumental analysis itself, particularly with regard to the carry-over of analytes from standards or highly contaminated samples into other analyses.

Internal Standard – Internal standards are organic compounds which are similar to the analytes of interest, but which are not found in the samples. The chosen internal standards are used to help

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calibrate the instrument's response and to compensate for slightly different injection amounts onto the instrument.

Laboratory Control Samples (LCS) – The LCS is an aliquot of analyte free water or analyte free solid to which known amounts target analytes are added. The LCS is prepared and analyzed in exactly the same manner as the samples. The percent recovery is compared to established limits and assists in determining whether the batch is in control.

4. INTERFERENCES

4.1. Solvents, reagents, glassware, and other sample processing equipment may yield discrete artifacts and/or elevated baselines causing misinterpretation of gas chromatograms. All of these materials must be demonstrated to be free from interferences, under the conditions of the analysis, by analyzing method blanks. Specific selection of reagents and the use of solvents purified by distillation in all-glass systems may be required. Analysts should pay particular attention to the introduction of contamination from phthalate esters and use sample handling practices which minimize the introduction of the compounds into extracts.

4.2. Interferences co-extracted from samples will vary considerably from source to source, depending upon the sample matrix. Cleanup techniques are generally required to eliminate significant levels of interferences. The presence of organochlorine pesticides in the sample can represent a potential interference which may exist after cleanup steps have been taken.

5. SAFETY

5.1. All appropriate safety precautions for handling solvents, reagents and samples must be taken when performing this procedure. This includes the use of personnel protective equipment, such as, safety glasses, lab coat and the correct gloves.

5.2. Chemicals, reagents and standards must be handled as described in the CAS safety policies, approved methods and in MSDSs where available. Refer to the CAS Environmental, Health and Safety Manual and the appropriate MSDS prior to beginning this method.

5.3. The toxicity or carcinogenicity of each compound or reagent used in this method may not be precisely determined; however, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level.

6. SAMPLE COLLECTION, CONTAINERS, PRESERVATION, AND STORAGE

6.1. Containers used to collect samples for the determination of semivolatile organic compounds should be soap and water washed followed by methanol (or isopropanol) rinsing. The sample containers should be of glass or teflon and have screw-top covers with teflon liners. In situations where teflon is not available, solvent-rinsed aluminum foil may be used as a liner. Highly acidic or basic samples may react with the aluminum foil, causing eventual

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contamination of the sample. Plastic containers or lids may not be used for the storage of samples due to the possibility of sample contamination from the phthalate esters and other hydrocarbons within the plastic.

6.2. Sample containers should be filled with care so as to prevent any portion of the collected sample coming in contact with the sampler's gloves, thus causing contamination. Samples should not be collected or stored in the presence of exhaust fumes. If the sample comes in contact with the sampler (e.g., if an automatic sampler is used), run reagent water through the sampler and use the rinseate as a field blank.

6.3. Water and soil samples must be iced or refrigerated at 4 ± 2ºC from time of collection until extraction. Tissue samples should be stored in accordance with project requirements, typically refrigerated or frozen. Water and soil samples must be iced or refrigerated at 4º C from time of collection until extraction.

6.4. There are no holding time requirements for this method.

7. APPARATUS AND EQUIPMENT

7.1. Gas Chromatograph System

7.1.1. An analytical system complete with a temperature-programmable gas chromatograph suitable for splitless or on-column injection and all required accessories, including syringes, analytical columns, and gases.

7.1.2. Gas Chromatograph - Equipped with cool-on-column or split/splitless injection port that is temperature programmable with dual ECD, HP/AGILENT 5890 or 6890. See Table 2 for typical chromatographic conditions.

7.1.3. GC Autosampler: The GC system should be configured with a compatible autosampler for automated injection of standards, samples, and QC samples.

7.1.4. Columns :
Column 1: 40 meter Restek RTX-5, 0.18mm ID, 0.20 µm, or equivalent.
Column 2: 40 meter Restek RTX-Dioxin, 0.18mm ID, 0.18 µm, or equivalent.

7.1.5. Data System - A computer system must be interfaced to the GC/ECD. The system must allow the continuous acquisition and storage on machine-readable media of all chromatographic data obtained throughout the duration of the chromatographic program. The computer must have software that performs analysis of chromatographic data (response vs. time) and allows the user to program qualitative and quantitative parameters which result in analyte identification and quantitation. The most recent version of the manufacturer’s software is preferred (HP Chemstation/Enviroquant).

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7.2. Analytical Balance (0.0001 g), syringes, bottles, and vials as required. All bottles and vials should use Teflon-lined caps or septa.

7.3. Sample preparation and cleanup apparatus - Glassware and associated apparatus required to perform sample extraction and cleanup is described in the applicable extraction and cleanup SOPs.

8. STANDARDS, REAGENTS, AND CONSUMABLE MATERIALS

8.1. Solvents: Methylene chloride, hexane, acetone, methanol, or other solvents as appropriate. Pesticide quality or equivalent.

8.2. Stock Standard Solutions

8.2.1. Stock standard solutions may be purchased from a number of vendors. All reference standards, where possible, must be traceable to SI units or NIST certified reference materials.

8.2.2. Two custom mixes of congeners are purchased from Accustandard 10 µg/mL in iso-octane (PCB 1 is at 100ug/ml). Equivalent standards may be purchased from other approved vendors. Congeners contained in each of the calibration mixes are listed below.

Mix A		**Mix B**	
PCB 8	PCB 138	PCB 1	PCB 166
PCB 18	PCB 141	PCB 5	PCB 168
PCB 28	PCB 151	PCB 31	PCB 169
PCB 33	PCB 156	PCB 37	PCB 170
PCB 44	PCB 167	PCB 49	PCB 174
PCB 52	PCB 177	PCB 60	
PCB 56	PCB 180	PCB 77	
PCB 66	PCB 183	PCB 81	
PCB 70	PCB 184	PCB 90	
PCB 74	PCB 187	PCB 95	
PCB 87	PCB 189	PCB 105	
PCB 97	PCB 194	PCB 118	
PCB 99	PCB 195	PCB 119	
PCB 101	PCB 201	PCB 126	
PCB 110	PCB 203	PCB 128	
PCB 114	PCB 206	PCB 149	
PCB 123	PCB 209	PCB 153	
PCB 132		PCB 157	
		PCB 158	

8.2.3. Transfer the stock standard solutions into Teflon-sealed screw-cap bottles. Store at 4°C and protect from light. Stock standards should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards

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from them. Stock standard solutions must be replaced after one year, or as specified by the manufacturer.

8.2.4. ICV Standards - Two custom mixes of congeners (same congeners as calibration stock) are purchased from Ultra Scientific 10 µg/mL in iso-octane. Equivalent standards may be purchased from other approved vendors as long as they are a source other than the calibration stocks. These solutions are diluted to give working ICV standards that are mid-range in the calibration curve.

8.3. Calibration Standards: Using the two stock standards, two sets of calibration standards are prepared at a minimum of five concentration levels by diluting in hexane or isooctane. One of the concentration levels should be at the method reporting limit (MRL). The remaining concentration levels should correspond to the expected range of concentrations found in real samples or should define a linear range. Typical concentrations are 2.5, 5, 10, 50, 100, and 200 ppb. Internal standard is added at 100 ppb. Calibration solutions must be monitored closely for signs of degradation. The recommended replacement interval is 6 months.

8.4. Surrogate Standard: The performance of the extraction, cleanup, and analytical system and the effectiveness of the method for each sample matrix are monitored by spiking each sample, method blank, and QC sample with a surrogate compound. For this analysis, the surrogate is tetrachloro-m-xylene (TCMX). Prepare a spiking solution at 4 ug/ml. Store at 4°C and protect from light. Surrogate spiking solutions are replaced every six months.

8.5. Matrix Spike Standards: Matrix spike solutions are prepared at a level representative of the calibration mid-range. Prepare a solution that contains all QC target analytes. Either a subset of the target list or the entire target list is reported depending on project requirements. Store at 4±2°C and protect from light. Matrix spike solutions are replaced every six months.

8.6. Internal Standard Solution: The internal standard used is 2,4-Dibromobiphenyl. Prepare a working standard at a concentration of 10ug/ml. For each 100 ul of extract, spike with 1 ul of internal standard solution resulting in a concentration of 100ng/ml (i.e. 0.5 ml of extract is spiked with 5 ul and 1.0 ml of extract is spiked with 10 ul).

9. PREVENTIVE MAINTENANCE

9.1. All maintenance activities are recorded in a maintenance logbook kept for each instrument. Pertinent information (serial numbers, instrument I.D., etc.) must be in the logbook. This includes the routine maintenance described in section 9. The entry in the log must include: date of event, the initials of who performed the work, and a reference to analytical control.

9.2. Carrier gas - Inline purifiers or scrubbers should be in place for all sources of carrier gas. These are selected to remove water, oxygen, and hydrocarbons. Purifiers should be changed as recommended by the supplier.

9.3. Gas Chromatograph

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9.3.1. Whenever GC maintenance is performed, care should be taken to minimize the introduction of air or oxygen into the column. Injection port maintenance includes changing the injection port liner, seal, washer, o-ring, septum, column ferrule, and autosampler syringe as needed. Liners and seals should be changed when recent sample analyses predict a problem with chromatographic performance. In some cases liners and seals may be cleaned and re-used.

9.3.2. Clipping off a small portion of the head of the column often improves chromatographic performance. When cutting off any portion of the column, make sure the cut is straight and "clean" (uniform, without fragmentation) by using the proper column cutting tool.

9.3.3. Over time, the column will exhibit poorer overall performance, as contaminated sample matrices are analyzed. The length of time for this to occur will depend on the samples analyzed. When a noticeable decrease in column performance is evident and other maintenance options do not result in improvement, the column should be replaced. This is especially true when evident in conjunction with calibration difficulties.

10. RESPONSIBILITIES

10.1. It is the responsibility of the analyst to perform the analysis according to this SOP and to complete all documentation required for data review. Analysis and interpretation of the results are performed by personnel in the laboratory who have demonstrated the ability to generate acceptable results utilizing this SOP. This demonstration is in accordance with the training program of the laboratory. Final review and sign-off of the data is performed by the department supervisor/manager or designee.

10.2. It is the responsibility of the department supervisor/manager to document analyst training. Documenting method proficiency, as described in the SOP for Documentation of Training, is also the responsibility of the department supervisor/manager.

11. PROCEDURE

11.1. Water samples are extracted at a neutral pH (5-9) with methylene chloride using EPA Method 3520C. Sediment, soil, and solid samples, as well as tissue samples, are extracted using EPA Method 3540C or 3541. Refer to the CAS SOPs for those procedures. Sample cleanup procedures may be used as appropriate for the samples. See Figure 1.

11.2. Calibration

The calibration procedure(s) and options chosen must follow the CAS protocols. Any exceptions to the calibration procedures detailed in the CAS SOP for *Calibration of Instruments for Organics Chromatographic Analyses* are described as follows:

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Note: Certain state or program protocols have specific procedures for calibration. The analyst must ensure that the correct procedures are used. Known exceptions are as follows:

- The use of quadratic regression calibration is not allowed for projects (samples) originating from South Carolina and under the SC DHEC lab certification.

11.2.1. The GC is configured to perform simultaneous analysis using the two columns and dual ECD detectors. Operating conditions must be the same for all calibration analyses and related sample analyses. The recommended GC operating conditions are given in Table 2.

11.2.2. Two separate sets of calibration runs are performed to generate the complete initial calibration, one for each congener standard mix. Care must be taken when calibrating due to known co-elutions between congeners contained in the different mixes.

11.2.3. Initial Calibration

11.2.3.1. For each congener standard mix, calibration standards at a minimum of 5 concentration levels are analyzed. Analyze an aliquot of each calibration standard and tabulate the peak area and concentration for each compound. Calibration curves are created using internal standard procedures. Refer to the CAS SOP for *Calibration of Instruments for Organics Chromatographic Analyses* for detailed calibration and evaluation procedures.

11.2.3.2. The initial calibration is verified by an independent source or lot. Prepare an independent calibration verification standard (ICV) by dilution of a stock solution purchased from a different vendor (or prepared by another analyst if a 2^{nd} source is not available) and analyze immediately after each initial calibration. Calculate the concentration using the typical procedure used for quantitation. Calculate the percent difference (%D) from the ICV true value. Evaluate the ICV as described in the *SOP for Calibration of Instruments for Organics Chromatographic Analyses*.

11.3. Retention Time Windows

11.3.1. Establish retention time windows with the GC system in acceptable operating conditions. Make three injections of all analytes throughout the course of a 72-hour period. Serial injections over less than a 72-hour period may result in retention time windows that are too tight. Using retention times from these analyses, calculate retention time windows. Refer to EPA Method 8000C for detailed instructions.

11.3.2. Plus or minus three times the standard deviation of the absolute retention times for each standard will be used to define the retention time window; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

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In those cases where the standard deviation for a particular standard is zero, substitute the standard deviation of a close eluting, similar compound to develop a valid retention time window.

11.3.3. Calculate retention time windows for each standard on each GC column and whenever a new GC column is installed. Retain this data in the method file.

11.4. Calibration Verification

11.4.1. The working calibration curve or calibration factor must be verified on each analytical sequence by the analysis of one or more mid-range calibration standards (CCV). A mid-level standard (CCV) must be injected at the start of each sequence. For internal standard calibration technique, a CCV standard is to be analyzed every 12 hours or 20 samples, whichever comes first. The analysis of a closing CCV is not required with internal standard calibration methods.

Note: DoD projects require a CCV analysis every 10 field samples.

11.4.2. Compare the response factor with the average response factor from the initial calibration. The acceptance criteria for all analytes in the CCV are a response (RF or concentration) within ± 20% D of the expected value, as compared to the initial calibration. If a calibration model other than average RF is used, compare the calculated result to the true value rather than using RF. Refer to the SOP for *Calibration of Instruments for Organics Chromatographic Analyses* for CCV evaluation protocols.

11.5. Sample Analysis

11.5.1. Add the internal standard spiking solution to each extract to obtain the nominal extract concentration of 100ng/ml. Analyze the extract using the recommended operating conditions specified in Table 1, ensuring that the same conditions are used for samples as for calibration. Dilution(s) may be made prior to analysis.

11.5.2. Perform all qualitative and quantitative measurements as described in the following sections. Store the extracts at 4 ± 2° C, protected from light in vials equipped with unpierced Teflon lined septa.

12. QA/QC REQUIREMENTS

12.1. Initial Precision and Recovery Validation

12.1.1. The accuracy and precision of the procedure must be validated before analyses of samples begin, or whenever significant changes to the procedures have been made. To do this, four water samples are spiked with the LCS spike solution, then prepared and analyzed.

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12.2. Method Detection Limits and Method Reporting Limits

12.2.1. A method detection limit (MDL) study must be undertaken before analysis of samples can begin. To establish detection limits that are precise and accurate, the analyst must perform the following procedure. Spike seven blank matrix (water or soil) samples with MDL spiking solution at a level below the MRL. Follow the analysis procedures in Section 11 to analyze the samples.

12.2.2. Calculate the average concentration found (x) in µg/mL, and the standard deviation of the concentrations (s) in µg/mL for each analyte. Calculate the MDL for each analyte. Refer to the CAS *SOP Performing Method Detection Limit Studies and Establishing Limits of detection and Quantification (ADM-MDL).* The MDL study must be verified annually.

12.2.3. Limits of Quantification (LOQ)

12.2.3.1. The laboratory establishes a LOQ for each analyte as the lowest reliable laboratory reporting concentration or in most cases the lowest point in the calibration curve which is less than or equal to the desired regulatory action levels, based on the stated project requirements. Analysis at the lowest point calibration standard provides confirmation of the established sensitivity of the method. The LOQ recoveries should be within 50% of the true values to verify the data reporting limit. Refer to the CAS SOP *Performing Method Detection Limit Studies and Establishing Limits of detection and Quantification (ADM-MDL)*

12.2.4. The Method Reporting Limits (MRLs) used at CAS are the routinely reported lower limits of quantitation which take into account day-to-day fluctuations in instrument sensitivity as well as other factors. These MRLs are the levels to which CAS routinely reports results in order to minimize false positive or false negative results. The MRL is normally two to ten times the method detection limit.

12.3. Ongoing QC Samples required are described in the CAS-Kelso Quality Assurance Manual and in the SOP for Sample Batches. Additional QC Samples may be required in project specific quality assurance plans (QAPP). For example projects managed under the DoD ELAP must follow requirements defined in the DoD *Quality Systems Manual for Environmental Laboratories.* General QA requirements for DoD QSM are defined in the laboratory SOP, Department *of Defense Projects – Laboratory Practices and Project Management (ADM-DOD).* General QC Samples are:

12.3.1. Method Blank

12.3.1.1. A method blank is extracted and analyzed with every batch of 20 samples, or daily, whichever is more frequent, to demonstrate that there are no method

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interferences. If the method blank shows any hits above the reporting limit, corrective action must be taken. Corrective action includes recalculation, reanalysis, system cleaning, or reextraction and reanalysis.

Note: DoD projects require that no analyte be detected > ½ the RL or 1/10 the regulatory limit, whichever is greater.

12.3.2. Laboratory Control Sample

12.3.2.1. A laboratory control sample (LCS) must be extracted and analyzed with every batch of 20 samples, or daily, whichever is more frequent. The LCS is prepared by adding a known amount of the matrix spike solution to a blank sample matrix, then proceeding preparation and analysis. Calculate the % recovery as follows:

$$\%R = X/TV \times 100$$

Where X = Concentration of the analyte recovered
TV = True value of amount spiked

12.3.2.2. Acceptance criteria for lab control samples are listed in Table 2. Project-specific or program-specific acceptance criteria may supersede CAS criteria. For example, for samples requiring South Carolina DHEC certification the acceptance criteria are 70-130 % recovery. If the lab control sample (LCS) fails acceptance limits for any of the compounds, the analyst must evaluate the system and calibration. If no problems are found, corrective action must be taken. The acceptance criteria listed are current criteria, but are subject to change as control limits are updated.

12.3.3. Matrix Spike

12.3.3.1. A matrix spike (MS) and duplicate matrix spike (DMS) must be extracted and analyzed with every batch of 20 samples. The MS/DMS is prepared by adding a known amount of the matrix spike solution to the sample then proceeding with preparation and analysis. Calculate percent recovery (%R) as:

$$\%R = \frac{X - X1}{TV} \times 100$$

Where X = Concentration of the analyte recovered
X1 = Concentration of unspiked analyte
TV = True value of amount spiked

Calculate Relative Percent Difference (RPD) as:

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$$RPD = \frac{|R1 - R2|}{(R1 + R2)/2} x\,100$$

Where R1 = %recovery of the MS
R2 = %recovery of the DMS

12.3.3.2. The acceptance limits for the MS/DMS are given in Table 2. If the MS/DMS recovery is out of acceptance limits for reasons other than matrix effects, corrective action must be taken. Corrective action includes recalculation, reanalysis, or reextraction and reanalysis.

Note: DoD projects require a matrix spike or sample duplicate with each preparation batch of samples. The RPD should be ≤ 30%.

12.3.4. Surrogate

12.3.4.1. Surrogate spike is added to every sample, blank and spike prior to extraction. Add a known amount of the surrogate spike to all samples and QC samples in the extraction set. The surrogate is used to monitor extraction efficiency. Calculate surrogate percent recovery (%R) as:

$$\%R = S/V \times 100$$

Where S = The amount of surrogate recovered
V = The amount spiked/final volume

12.3.4.2. The surrogate acceptance limits are given in Table 2. If the surrogate recovery is outside of acceptance limits for reasons other than matrix effects, corrective action must be taken. Corrective actions include recalculation, reanalysis, or reextraction and reanalysis.

12.3.5. Internal Standard

Compare the internal standard response in CCVs and samples to that from the initial calibration. The criterion is 50-200% of the average of responses from the initial calibration standards.

13. DATA REDUCTION, REVIEW, AND REPORTING

13.1. Qualitative Analysis

13.1.1. The sample component RRT must compare within ± 0.03 RRT units of the RRT for the compound in the opening CCV. Care must be taken when reporting samples where congeners contained in both mixes have been identified, due to known co-elutions between congeners contained in the different mixes.

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13.1.2. Confirmation of all tentative hits must be made. Confirmation is made by injecting the sample extract on two columns with dissimilar phases simultaneously. If the retention time matches on both columns, then the hit for the analyte is considered a confirmed hit. As further confirmation, subsequent quantitations on the two columns should agree. Refer to the CAS Confirmation Policy for guidance. If on one column, known coeluting compounds exist, a quantitation difference may not be an appropriate measure of confirmation. In the case of known coeluting compounds on one column, using quantitation on the two columns as a measure of confirmation should take into account the relative amounts of the two components. When pesticides are known to be present, the quantitations on the two columns may not agree within 40%D and the analyst's judgement should be used to determine if the results represent a positive *congener* result at that quantitation level. Confirmation by GCMS may also be used if the concentration is high enough for detection and accurate spectral identification. A GCMS confirmation need only be qualitative in nature.

13.2. Quantitative Analysis

13.2.1. When a compound has been identified, the quantitation of the compound is based on the area of the compound identified. The quantitation technique used is the same as the calibration technique for the specific component.

13.2.2. Internal Standard quantitation.

Calculate the concentration of each identified analyte in the sample as follows:

Water: $$Concentration\,(\mu g/L) = \frac{(A_x)(I_s)(V_t)}{(A)_{is}(RF)(V_o)(V_i)}$$

where:
- Ax = Area of the compound being measured.
- Is = Amount of internal standard injected (ng).
- Vt = Volume of total extract, taking into account dilutions (i.e. a 1-to-10 dilution of a 1 ml extract will mean a Vt = 10,000 µL).
- Ais = Area of the internal standard.
- RF = Average response factor for compound being measured.
- Vo = Volume of water extracted (ml).
- Vi = Volume of extract injected (µL).

Sediment/Soil Sludge (on a dry-weight basis) and Tissue (on a wet-weight basis):

$$Concentration\,(\mu g/kg) = \frac{(Ax)(I_s)(V_t)}{(A_{is})(RF)(V_i)(W_s)(D)}$$

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where: Ax, Is, Vt, Ais, RF, Vi = Same as for water.
Ws = Weight of sample extracted or diluted in grams.
D = % dry weight of sample/100, or 1 for a wet-weight basis.

13.2.3. Quadratic Curve quantitation:

Using the peak area for the identified component, use the calibration curve constructed during initial calibration to determine the solution (extract) concentration. Use the solution concentration to determine sample concentration as follows:

Water: $$Concentration\ (\mu g / L) = \frac{(Cex)\ (Vf)\ (D)}{(Vs)}$$

where:

Cex = Calculated concentration in the extract analyzed (ug/mL)
Vf = Final volume of extract (mL).
D = Dilution factor.
Vs = Volume of sample extracted (L).

Sediment/Soil Sludge (on a dry-weight basis) and tissue (on a wet-weight basis):

$$Concentration\ (ng / g) = \frac{(Cex)\ (Vf)\ (D)}{(W)}$$

where: Cex = Calculated concentration in the extract analyzed (ng/uL)
Vf = Final volume of extract (uL).
W = Weight of sample extracted or diluted in grams.
D = % dry weight of sample/100, or 1 for a wet-weight basis.

13.2.4. If the quantitation result is over the calibration range for any compound, a dilution re-analysis is required.

13.3. Data Review

Data for the analytical sequence is compiled in a data "pack" which includes all calibration, QC, and sample data for the sequence. Following primary data interpretation and calculations, all data is reviewed by a secondary analyst. Following generation of the report, the report is also reviewed. Refer to the *SOP for Laboratory Data Review Process* for details.

13.4. Reporting

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13.4.1. Reports are generated using the STEALTH data reporting system, which compiles the SMO Login information. This compilation is then transferred to a file which STEALTH uses to generate a report. The forms generated may be CAS standard reports, DOD, or client-specific reports. The compiled data from LIMS is also used to create EDDs.

13.4.2. As an alternative, reports are generated using Excel© templates located in R:\SVG\forms. The analyst should choose the appropriate form and QC pages to correspond to required tier level and deliverables requirements. The detected analytes, surrogate and matrix spikes are then transferred, by hand or electronically, to the templates.

14. CONTINGENCIES FOR HANDLING OUT-OF-CONTROL OR UNACCEPTABLE DATA

14.1. Refer to the SOP for *Corrective Action* for procedures for corrective action. Personnel at all levels and positions in the laboratory are to be alert to identifying problems and nonconformities when errors, deficiencies, or out-of-control situations are detected.

14.2. Handling out-of-control or unacceptable data

14.2.1. On-the-spot corrective actions that are routinely made by analysts and result in acceptable analyses should be documented as normal operating procedures, and no specific documentation need be made other than notations in laboratory maintenance logbooks, runlogs, for example.

14.2.2. Documentation of a nonconformity must be done using a Nonconformity and Corrective Action Report (NCAR) when:

- Corrective action is not taken or not possible
- Corrective action fails to correct an out-of-control problem on a laboratory QC or calibration analysis.
- Reanalysis corrects the nonconformity but is not a procedurally compliant analysis.

15. METHOD PERFORMANCE

15.1. This method was validated through single laboratory studies of accuracy and precision. Refer to the reference method for additional method performance data available.

15.2. Method Reporting Limits are established for this method based on MDL studies and as specified in the CAS SOP *Performing Method Detection Limit Studies and Estimation of Limits of Detection and Quantitation (ADM-MDL).*

16. POLLUTION PREVENTION

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It is the laboratory's practice to minimize the amount of solvents, acids and reagent used to perform this method wherever feasible. Standards are prepared in volumes consistent with methodology and only the amount needed for routine laboratory use is kept on site. The threat to the environment from solvent and reagents used in this method can be minimized when recycled or disposed of properly.

17. WASTE MANAGEMENT

17.1. The laboratory will comply with all Federal, State and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions as specified in the CAS EH&S Manual.

17.2. This method uses non-halogenated solvents and any waste generated from this solvent must be placed in the collection cans in the lab. The solvent will then be added to the hazardous waste storage area and disposed of in accordance with Federal and State regulations.

18. TRAINING

18.1. A minimum training outline is given below. Also, refer to the generic training plan given in the *SOP for Documentation of Training*.

18.2. Review literature (see references section). Read and understand the SOP. Also review the applicable MSDS for all reagents and standards used. Following these reviews, observe the procedure as performed by an experienced analyst at least three times.

18.3. The next training step is to assist in the procedure under the guidance of an experienced analyst for a period of time. During this period, the analyst is expected to transition from a role of assisting, to performing the procedure with minimal oversight from an experienced analyst.

18.4. Perform initial precision and recovery (IPR) study as described above for water samples. Summaries of the IPR are reviewed and signed by the supervisor. Copies may be forwarded to the employee's training file. For applicable tests, IPR studies should be performed in order to be equivalent to NELAC's Initial Demonstration of Capability.

18.5. Training is documented following the *SOP for Documentation of Training.*

NOTE: When the analyst training is documented by the supervisor on internal training documentation forms, the supervisor is acknowledging that the analyst has read and understands this SOP and that adequate training has been given to the analyst to competently perform the analysis independently.

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19. METHOD MODIFICATIONS

19.1. There are no known modifications in this laboratory standard operating procedure from the reference method.

20. REFERENCES

20.1. Polychlorinated Biphenyls (PCBs) as Aroclors, Method 8082A, Revision 1, February 2007, EPA Test Methods for Evaluating Solid Waste, SW-846, Update IV

20.2. Determinative Chromatographic Separations, EPA SW846, Test Methods For Evaluating Solid Waste, On-Line, Method 8000C, Revision 3, March 2003.

20.3. Extraction Methods 3510C (Separatory Funnel Liquid-Liquid Extraction), 3520C (Continuous Liquid-Liquid Extraction), 3540C (Soxhlet Extraction), 3545 (Pressurized Fluid Extraction), and 3550B (Ultrasonic Extraction); EPASW846, Test Methods For Evaluating Solid Waste, Update III, December, 1996.

20.4. Cleanup Methods 3610B (Alumina Cleanup), 3630C (Silica Gel Cleanup), 3640A (Gel-Permeation Cleanup), 3665A (Sulfuric Acid / Permanganate Cleanup); EPASW846, Test Methods For Evaluating Solid Waste, Updates II and III, September 1994 and December 1996.

20.5. *DoD Quality systems Manual for Environmental Laboratories* Version 4.1 4/22/2009

21. CHANGES SINCE THE LAST REVISION

21.1. Sec 1.2 removed PQL is not equal to MRL
21.2. Sec 3 added definition for LCS
21.3. Sec 3 ‘and varying ion suppression in the source’ was removed from the Internal Standard definition
21.4. Sec 6.4 removed HT requirements to comply with SW-846 table 4-1
21.5. Sec 8.2.1 is new
21.6. Sec 8.2.2 removed first sentence that was in previous revision
21.7. Sec 8.2.4 removed first sentence that was in previous revision
21.8. Sec 9.1 updated to comply with current standards
21.9. Sec 11.2.3.1 ‘Analyze 1 µL’ was replaced with ‘Anaylize an aliquot’
21.10. Sec 19 is new

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FIGURE 1

CONGENER-SPECIFIC DETERMINATION OF POLYCHLORINATED BIPHENYLS (PCBS)

SAMPLE CLEANUP FLOWCHART

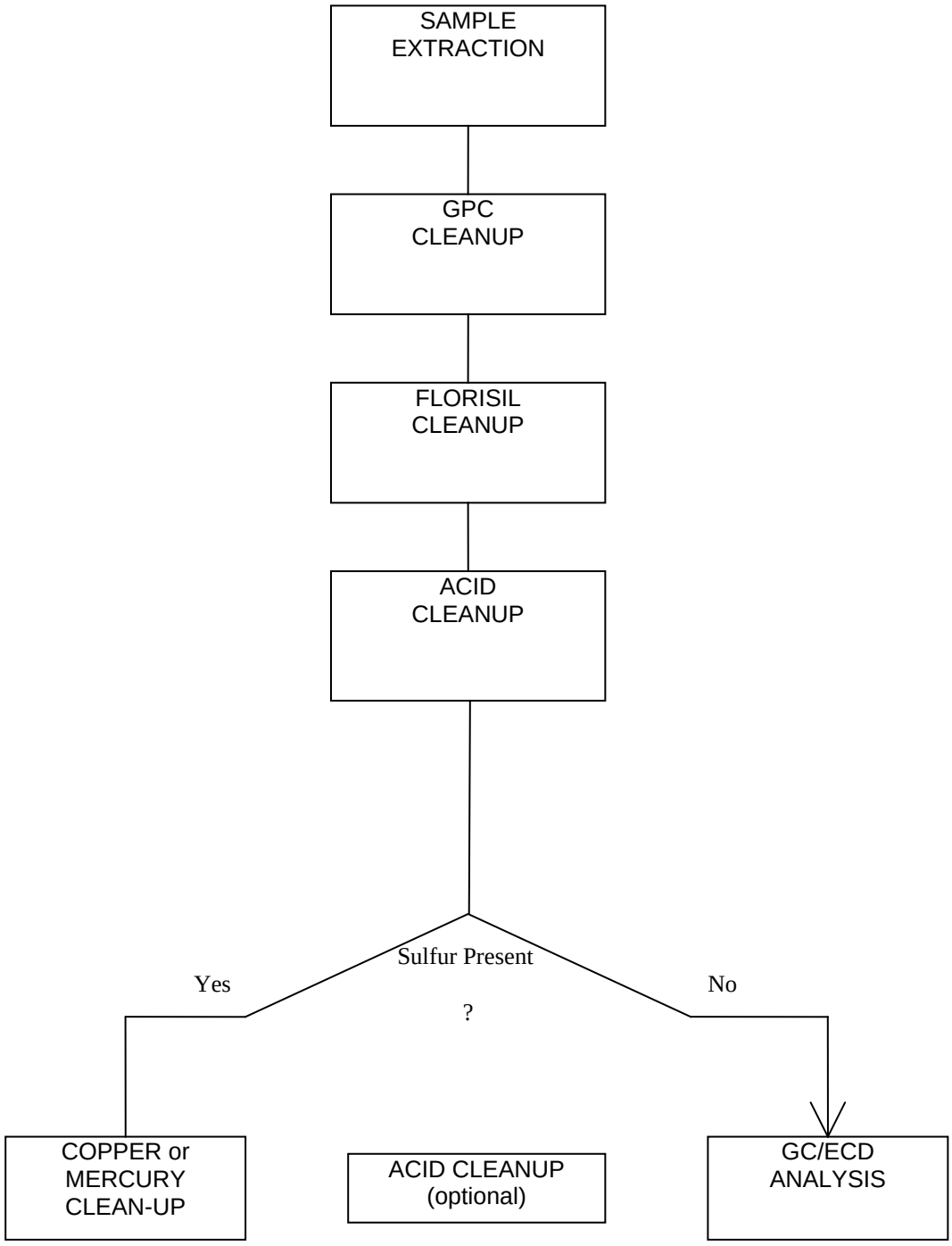

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TABLE 1

Gas Chromatograph Operating Conditions*

Gas Chromatograph:	Hewlett-Packard Model 6890 or equivalent w/micro ECD
Injection Port Temperature:	PTV: 40°C for 0 min., 250° /min to 250°C, hold 5 min., 250° /min to 325°C, hold 5 min.
Oven Temperature Program:	40°C for 0.5 minute, 20°/min ramp to 200°C, hold 1 min., ramp 5°/minute to 280° C, hold for 0 minutes, ramp 5°/minute to 310° C, hold for 0.25 minutes.
Detector Temperature:	325 C
Injection Volume:	1µL, split 1:10, split flow 26.1mL/min.
Column:	40 m, RTX-5 and 40 m RTX-Dioxin.
Carrier Gas:	Helium, programmed: 1.3mL/min constant flow
Auxillary Gas:	Nitrogen
Data System:	HP Enviroquant

The instrument temperatures may be modified depending on the instrument used. Also, the GC column diameter and film thickness depend on the instrument used. All conditions must be the same for initial calibration, continuing calibration, sample, and QC analyses.

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TABLE 2

CAS/KELSO DATA QUALITY OBJECTIVES

METHOD	ANALYTE	CAS No.	MATRIX	MDLa	MRL	LODb	LOQc	UNITS	Accuracy (LCS %Rec.)	Matrix Spike (%Rec.)	Precision (% RPD)
8082 Congeners	PCB1 (2-Chlorobiphenyl)	2051-60-7	Water	1.1	5	10	50	ng/L	70-130	70-130	30
8082 Congeners	PCB101 (2,2',4,5,5'-Pentachlorobiphenyl)	37680-73-2	Water	0.23	5	0.75	5	ng/L	70-130	70-130	30
8082 Congeners	PCB105 (2,3,3',4,4'-Pentachlorobiphenyl)	32598-14-4	Water	0.35	5	1	5	ng/L	43-135	43-135	30
8082 Congeners	PCB110 (2,3,3',4',6-Pentachlorobiphenyl)	38380-03-9	Water	0.19	5	0.75	5	ng/L	70-130	70-130	30
8082 Congeners	PCB114 (2,3,4,4',5-Pentachlorobiphenyl)	74472-37-0	Water	0.23	5	0.75	5	ng/L	50-125	50-125	30
8082 Congeners	PCB118 (2,3',4,4',5-Pentachlorobiphenyl)	31508-00-6	Water	0.32	5	0.25	5	ng/L	47-135	37-144	30
8082 Congeners	PCB119 (2,3',4,4',6-Pentachlorobiphenyl)	56558-17-9	Water	0.25	5	1	5	ng/L	70-130	70-130	30
8082 Congeners	PCB123 (2',3,4,4',5-Pentachlorobiphenyl)	65510-44-3	Water	0.2	5	0.75	5	ng/L	49-126	49-126	30
8082 Congeners	PCB126 (3,3',4,4',5-Pentachlorobiphenyl)	57465-28-8	Water	0.24	5	1	5	ng/L	44-135	42-126	30
8082 Congeners	PCB128 (2,2',3,3',4,4'-Hexachlorobiphenyl)	38380-07-3	Water	0.95	5	1	5	ng/L	37-129	37-117	30
8082 Congeners	PCB132 (2,2',3,3',4,6'-Hexachlorobiphenyl)	38380-05-1	Water	0.25	5	0.75	5	ng/L	70-130	70-130	30
8082 Congeners	PCB138 (2,2',3,4,4',5'-Hexachlorobiphenyl)	35065-28-2	Water	0.23	5	1.75	5	ng/L	49-118	56-103	30
8082 Congeners	PCB141 (2,2',3,4,5,5'-Hexachlorobiphenyl)	52712-04-6	Water	0.23	5	0.75	5	ng/L	70-130	70-130	30
8082 Congeners	PCB149 (2,2',3,4',5',6-Hexachlorobiphenyl)	38380-04-0	Water	0.29	5	1	5	ng/L	70-130	70-130	30
8082 Congeners	PCB151 (2,2',3,5,5',6-Hexachlorobiphenyl)	52663-63-5	Water	0.21	5	0.75	5	ng/L	70-130	70-130	30
8082 Congeners	PCB153 (2,2',4,4',5,5'-Hexachlorobiphenyl)	35065-27-1	Water	0.51	5	1	5	ng/L	29-133	38-118	30
8082 Congeners	PCB156 (2,3,3',4,4',5-Hexachlorobiphenyl)	38380-08-4	Water	0.29	5	0.75	5	ng/L	54-137	41-140	30
8082 Congeners	PCB157 (2,3,3',4,4',5'-Hexachlorobiphenyl)	69782-90-7	Water	0.32	5	0.25	5	ng/L	27-153	27-153	30
8082 Congeners	PCB158 (2,3,3',4,4',6-Hexachlorobiphenyl)	74472-42-7	Water	0.21	5	1	5	ng/L	24-158	24-158	30
8082 Congeners	PCB166 (2,3,4,4',5,6-Hexachlorobiphenyl)	41411-63-6	Water	0.44	5	1	5	ng/L	70-130	70-130	30
8082 Congeners	PCB167 (2,3',4,4',5,5'-Hexachlorobiphenyl)	52663-72-6	Water	0.19	5	0.75	5	ng/L	45-124	45-124	30
8082 Congeners	PCB168 (2,3',4,4',5',6-Hexachlorobiphenyl)	59291-65-5	Water	0.27	5	1	5	ng/L	70-130	70-130	30
8082 Congeners	PCB169 (3,3',4,4',5,5'-Hexachlorobiphenyl)	32774-16-6	Water	0.41	5	1	5	ng/L	32-161	36-136	30
8082 Congeners	PCB170 (2,2',3,3',4,4',5-Heptachlorobiphenyl)	35065-30-6	Water	0.28	5	0.25	5	ng/L	36-135	44-121	30
8082 Congeners	PCB174 (2,2',3,3',4,5,6'-Heptachlorobiphenyl)	38411-25-5	Water	0.95	5	0.25	5	ng/L	70-130	70-130	30
8082 Congeners	PCB177 (2,2',3,3',4',5,6-Heptachlorobiphenyl)	52663-70-4	Water	0.19	5	0.75	5	ng/L	70-130	70-130	30
8082 Congeners	PCB18 (2,2',5-Trichlorobiphenyl)	37680-65-2	Water	0.95	5	1.75	5	ng/L	39-118	12-142	30

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8082 Congeners	PCB180 (2,2',3,4,4',5,5'-Heptachlorobiphenyl)	35065-29-3	Water	0.35	5	0.75	5	ng/L	55-137	61-122	30
8082 Congeners	PCB183 (2,2',3,4,4',5',6-Heptachlorobiphenyl)	52663-69-1	Water	0.2	5	1.75	5	ng/L	43-130	31-141	30
8082 Congeners	PCB184 (2,2',3,4,4',6,6'-Heptachlorobiphenyl)	74472-48-3	Water	0.19	5	0.75	5	ng/L	53-118	35-131	30
8082 Congeners	PCB187 (2,2',3,4',5,5',6-Heptachlorobiphenyl)	52663-68-0	Water	0.26	5	0.75	5	ng/L	55-117	51-116	30
8082 Congeners	PCB189 (2,3,3',4,4',5,5'-Heptachlorobiphenyl)	39635-31-9	Water	0.24	5	0.75	5	ng/L	54-130	54-130	30
8082 Congeners	PCB194 (2,2',3,3',4,4',5,5'-Octachlorobiphenyl)	35694-08-7	Water	1.6	5	0.75	5	ng/L	70-130	70-130	30
8082 Congeners	PCB195 (2,2',3,3',4,4',5,6-Octachlorobiphenyl)	52663-78-2	Water	0.29	5	0.75	5	ng/L	52-126	37-132	30
8082 Congeners	PCB201 (2,2',3,3',4,5',6,6'-Octachlorobiphenyl)	40186-71-8	Water	0.17	5	0.75	5	ng/L	70-130	70-130	30
8082 Congeners	PCB203 (2,2',3,4,4',5,5',6-Octachlorobiphenyl)	52663-76-0	Water	0.21	5	0.75	5	ng/L	70-130	70-130	30
8082 Congeners	PCB206 (2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl)	40186-72-9	Water	0.2	5	1.75	5	ng/L	53-123	52-119	30
8082 Congeners	PCB209 (Decachlorobiphenyl)	2051-24-3	Water	0.34	5	1.75	5	ng/L	53-124	41-127	30
8082 Congeners	PCB28 (2,4,4'-Trichlorobiphenyl)	7012-37-5	Water	5	5	0.75	5	ng/L	54-139	45-143	30
8082 Congeners	PCB31 (2,4',5-Trichlorobiphenyl)	16606-02-3	Water	0.39	5	3	5	ng/L	70-130	70-130	30
8082 Congeners	PCB33 (2',3,4-Trichlorobiphenyl)	38444-86-9	Water	0.84	5	1.75	5	ng/L	70-130	70-130	30
8082 Congeners	PCB37 (3,4,4'-Trichlorobiphenyl)	38444-90-5	Water	0.28	5	1	5	ng/L	70-130	70-130	30
8082 Congeners	PCB44 (2,2',3,5'-Tetrachlorobiphenyl)	41464-39-5	Water	0.35	5	0.75	5	ng/L	53-124	39-130	30
8082 Congeners	PCB49 (2,2',4,5'-Tetrachlorobiphenyl)	41464-40-8	Water	0.51	5	1	5	ng/L	70-130	70-130	30
8082 Congeners	PCB5 (2,3-Dichlorobiphenyl)	16605-91-7	Water	0.29	5	3	5	ng/L	70-130	70-130	30
8082 Congeners	PCB52 (2,2',5,5'-Tetrachlorobiphenyl)	35693-99-3	Water	0.67	5	0.75	5	ng/L	48-111	23-132	30
8082 Congeners	PCB56 (2,3,3',4'-Tetrachlorobiphenyl)	41464-43-1	Water	0.21	5	0.75	5	ng/L	70-130	70-130	30
8082 Congeners	PCB60 (2,3,4,4'-Tetrachlorobiphenyl)	25569-80-6	Water	0.26	5	1	5	ng/L	42-185	42-185	30
8082 Congeners	PCB66 (2,3',4,4'-Tetrachlorobiphenyl)	32598-10-0	Water	0.3	5	0.75	5	ng/L	51-118	51-114	30
8082 Congeners	PCB70 (2,3',4',5-Tetrachlorobiphenyl)	32598-11-1	Water	0.2	5	0.75	5	ng/L	70-130	70-130	30
8082 Congeners	PCB74 (2,4,4',5-Tetrachlorobiphenyl)	32690-93-0	Water	0.2	5	0.75	5	ng/L	70-130	70-130	30
8082 Congeners	PCB77 (3,3',4,4'-Tetrachlorobiphenyl)	32598-13-3	Water	0.25	5	1	5	ng/L	33-149	38-129	30
8082 Congeners	PCB8 (2,4'-Dichlorobiphenyl)	34883-43-7	Water	0.71	5	1.75	5	ng/L	44-127	25-133	30
8082 Congeners	PCB81 (3,4,4',5-Tetrachlorobiphenyl)	70362-50-4	Water	0.26	5	1	5	ng/L	22-161	22-161	30
8082 Congeners	PCB87 (2,2',3,4,5'-Pentachlorobiphenyl)	38380-02-8	Water	0.19	5	0.75	5	ng/L	52-112	38-121	30
8082 Congeners	PCB90 (2,2',3,4',5-Pentachlorobiphenyl)	68194-07-0	Water	1.1	5	1	5	ng/L	37-180	37-180	30
8082 Congeners	PCB95 (2,2',3,5',6-Pentachlorobiphenyl)	38379-99-6	Water	0.88	5	3	5	ng/L	70-130	70-130	30
8082 Congeners	PCB97 (2,2',3',4,5-Pentachlorobiphenyl)	41464-51-1	Water	0.32	5	1.75	5	ng/L	70-130	70-130	30
8082 Congeners	PCB99 (2,2',4,4',5-Pentachlorobiphenyl)	38380-01-7	Water	0.27	5	0.75	5	ng/L	70-130	70-130	30
8082 Congeners	Tetrachloro-m-xylene (Surr.)	877-09-8	Water	NA	NA	NA	NA	%	26-100	NA	NA

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CAS/KELSO DATA QUALITY OBJECTIVES cont.

METHOD	ANALYTE	CAS No.	MATRIX	MDLa	MRL	LODb	LOQc	UNITS	Accuracy (LCS %Rec.)	Matrix Spike (%Rec.)	Precision (% RPD)
8082 Congeners	PCB1 (2-Chlorobiphenyl)	2051-60-7	Soil	1.2	5.0	2.5	5	ug/kg	70-130	70-130	40
8082 Congeners	PCB101 (2,2',4,5,5'-Pentachlorobiphenyl)	37680-73-2	Soil	0.078	0.50	0.175	0.5	ug/kg	70-130	70-130	40
8082 Congeners	PCB105 (2,3,3',4,4'-Pentachlorobiphenyl)	32598-14-4	Soil	0.053	0.50	0.1	0.5	ug/kg	37-133	31-135	40
8082 Congeners	PCB110 (2,3,3',4',6-Pentachlorobiphenyl)	38380-03-9	Soil	0.082	0.50	0.088	0.5	ug/kg	70-130	70-130	40
8082 Congeners	PCB114 (2,3,4,4',5-Pentachlorobiphenyl)	74472-37-0	Soil	0.068	0.50	0.088	0.5	ug/kg	42-121	39-123	40
8082 Congeners	PCB118 (2,3',4,4',5-Pentachlorobiphenyl)	31508-00-6	Soil	0.078	0.50	0.1	0.5	ug/kg	34-151	28-152	40
8082 Congeners	PCB119 (2,3',4,4',6-Pentachlorobiphenyl)	56558-17-9	Soil	0.072	0.50	0.1	0.5	ug/kg	70-130	70-130	40
8082 Congeners	PCB123 (2',3,4,4',5-Pentachlorobiphenyl)	65510-44-3	Soil	0.092	0.50	0.175	0.5	ug/kg	42-126	48-119	40
8082 Congeners	PCB126 (3,3',4,4',5-Pentachlorobiphenyl)	57465-28-8	Soil	0.072	0.50	0.1	0.5	ug/kg	40-136	34-144	40
8082 Congeners	PCB128 (2,2',3,3',4,4'-Hexachlorobiphenyl)	38380-07-3	Soil	0.08	0.50	0.1	0.5	ug/kg	23-15	22-141	40
8082 Congeners	PCB132 (2,2',3,3',4,6'-Hexachlorobiphenyl)	38380-05-1	Soil	0.072	0.50	0.175	0.5	ug/kg	70-130	70-130	40
8082 Congeners	PCB138 (2,2',3,4,4',5'-Hexachlorobiphenyl)	35065-28-2	Soil	0.075	0.50	0.175	0.5	ug/kg	41-124	31-131	40
8082 Congeners	PCB141 (2,2',3,4,5,5'-Hexachlorobiphenyl)	52712-04-6	Soil	0.059	0.50	0.088	0.5	ug/kg	70-130	70-130	40
8082 Congeners	PCB149 (2,2',3,4',5',6-Hexachlorobiphenyl)	38380-04-0	Soil	0.07	0.50	0.263	0.5	ug/kg	70-130	70-130	40
8082 Congeners	PCB151 (2,2',3,5,5',6-Hexachlorobiphenyl)	52663-63-5	Soil	0.069	0.50	0.088	0.5	ug/kg	70-130	70-130	40
8082 Congeners	PCB153 (2,2',4,4',5,5'-Hexachlorobiphenyl)	35065-27-1	Soil	0.097	0.50	0.1	0.5	ug/kg	32-128	20-139	40
8082 Congeners	PCB156 (2,3,3',4,4',5-Hexachlorobiphenyl)	38380-08-4	Soil	0.073	0.50	0.088	0.5	ug/kg	45-133	40-132	40
8082 Congeners	PCB157 (2,3,3',4,4',5'-Hexachlorobiphenyl)	69782-90-7	Soil	0.076	0.50	0.1	0.5	ug/kg	34-134	41-133	40
8082 Congeners	PCB158 (2,3,3',4,4',6-Hexachlorobiphenyl)	74472-42-7	Soil	0.07	0.50	0.1	0.5	ug/kg	29-134	29-138	40
8082 Congeners	PCB166 (2,3,4,4',5,6-Hexachlorobiphenyl)	41411-63-6	Soil	0.061	0.50	0.1	0.5	ug/kg	70-130	70-130	40
8082 Congeners	PCB167 (2,3',4,4',5,5'-Hexachlorobiphenyl)	52663-72-6	Soil	0.088	0.50	0.175	0.5	ug/kg	35-131	39-122	40
8082 Congeners	PCB168 (2,3',4,4',5',6-Hexachlorobiphenyl)	59291-65-5	Soil	0.069	0.50	0.1	0.5	ug/kg	70-130	70-130	40
8082 Congeners	PCB169 (3,3',4,4',5,5'-Hexachlorobiphenyl)	32774-16-6	Soil	0.093	0.50	0.1	0.5	ug/kg	31-148	27-152	40
8082 Congeners	PCB170 (2,2',3,3',4,4',5-Heptachlorobiphenyl)	35065-30-6	Soil	0.066	0.50	0.1	0.5	ug/kg	28-134	19-144	40
8082 Congeners	PCB174 (2,2',3,3',4,5,6'-Heptachlorobiphenyl)	38411-25-5	Soil	0.08	0.50	0.1	0.5	ug/kg	70-130	70-130	40
8082 Congeners	PCB177 (2,2',3,3',4',5,6-Heptachlorobiphenyl)	52663-70-4	Soil	0.089	0.50	0.175	0.5	ug/kg	70-130	70-130	40
8082 Congeners	PCB18 (2,2',5-Trichlorobiphenyl)	37680-65-2	Soil	0.086	0.50	0.175	0.5	ug/kg	41-116	36-123	40
8082 Congeners	PCB180 (2,2',3,4,4',5,5'-Heptachlorobiphenyl)	35065-29-3	Soil	0.083	0.50	0.175	0.5	ug/kg	48-135	40-139	40
8082 Congeners	PCB183 (2,2',3,4,4',5',6-Heptachlorobiphenyl)	52663-69-1	Soil	0.064	0.50	0.175	0.5	ug/kg	34-134	30-141	40

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8082 Congeners	PCB184 (2,2',3,4,4',6,6'-Heptachlorobiphenyl)	74472-48-3	Soil	0.078	0.50	0.175	0.5	ug/kg	42-123	44-117	40
8082 Congeners	PCB187 (2,2',3,4',5,5',6-Heptachlorobiphenyl)	52663-68-0	Soil	0.087	0.50	0.175	0.5	ug/kg	38-141	31-139	40
8082 Congeners	PCB189 (2,3,3',4,4',5,5'-Heptachlorobiphenyl)	39635-31-9	Soil	0.068	0.50	0.088	0.5	ug/kg	43-129	51-121	40
8082 Congeners	PCB194 (2,2',3,3',4,4',5,5'-Octachlorobiphenyl)	35694-08-7	Soil	0.082	0.50	0.088	0.5	ug/kg	70-130	70-130	40
8082 Congeners	PCB195 (2,2',3,3',4,4',5,6-Octachlorobiphenyl)	52663-78-2	Soil	0.085	0.50	0.088	0.5	ug/kg	45-124	33-134	40
8082 Congeners	PCB201 (2,2',3,3',4,5',6,6'-Octachlorobiphenyl)	40186-71-8	Soil	0.13	0.50	0.088	0.5	ug/kg	70-130	70-130	40
8082 Congeners	PCB203 (2,2',3,4,4',5,5',6-Octachlorobiphenyl)	52663-76-0	Soil	0.077	0.50	0.088	0.5	ug/kg	70-130	70-130	40
8082 Congeners	PCB206 (2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl)	40186-72-9	Soil	0.078	0.50	0.088	0.5	ug/kg	45-125	39-130	40
8082 Congeners	PCB209 (Decachlorobiphenyl)	2051-24-3	Soil	0.085	0.50	0.088	0.5	ug/kg	45-128	38-131	40
8082 Congeners	PCB28 (2,4,4'-Trichlorobiphenyl)	7012-37-5	Soil	0.064	0.50	0.175	0.5	ug/kg	49-142	47-144	40
8082 Congeners	PCB31 (2,4',5-Trichlorobiphenyl)	16606-02-3	Soil	0.069	0.50	0.1	0.5	ug/kg	70-130	70-130	40
8082 Congeners	PCB33 (2',3,4-Trichlorobiphenyl)	38444-86-9	Soil	0.097	0.50	0.425	0.5	ug/kg	70-130	70-130	40
8082 Congeners	PCB37 (3,4,4'-Trichlorobiphenyl)	38444-90-5	Soil	0.12	0.50	0.1	0.5	ug/kg	70-130	70-130	40
8082 Congeners	PCB44 (2,2',3,5'-Tetrachlorobiphenyl)	41464-39-5	Soil	0.25	0.50	0.175	0.5	ug/kg	48-126	40-134	40
8082 Congeners	PCB49 (2,2',4,5'-Tetrachlorobiphenyl)	41464-40-8	Soil	0.095	0.50	0.1	0.5	ug/kg	70-130	70-130	40
8082 Congeners	PCB5 (2,3-Dichlorobiphenyl)	16605-91-7	Soil	0.2	0.50	0.263	0.5	ug/kg	70-130	70-130	40
8082 Congeners	PCB52 (2,2',5,5'-Tetrachlorobiphenyl)	35693-99-3	Soil	0.15	0.50	0.175	0.5	ug/kg	44-115	32-130	40
8082 Congeners	PCB56 (2,3,3',4'-Tetrachlorobiphenyl)	41464-43-1	Soil	0.12	0.50	0.175	0.5	ug/kg	70-130	70-130	40
8082 Congeners	PCB60 (2,3,4,4'-Tetrachlorobiphenyl)	33025-41-1	Soil	0.069	0.50	0.1	0.5	ug/kg	35-182	19-180	40
8082 Congeners	PCB66 (2,3',4,4'-Tetrachlorobiphenyl)	32598-10-0	Soil	0.1	0.50	0.088	0.5	ug/kg	44-121	29-136	40
8082 Congeners	PCB70 (2,3',4',5-Tetrachlorobiphenyl)	32598-11-1	Soil	0.15	0.50	0.175	0.5	ug/kg	70-130	70-130	40
8082 Congeners	PCB74 (2,4,4',5-Tetrachlorobiphenyl)	32690-93-0	Soil	0.1	0.50	0.175	0.5	ug/kg	70-130	70-130	40
8082 Congeners	PCB77 (3,3',4,4'-Tetrachlorobiphenyl)	32598-13-3	Soil	0.082	0.50	0.1	0.5	ug/kg	36-135	30-142	40
8082 Congeners	PCB8 (2,4'-Dichlorobiphenyl)		Soil	0.14	0.50	0.425	0.5	ug/kg	48-129	41-140	40
8082 Congeners	PCB81 (3,4,4',5-Tetrachlorobiphenyl)	70362-50-4	Soil	0.07	0.50	0.1	0.5	ug/kg	25-147	30-133	40
8082 Congeners	PCB87 (2,2',3,4,5'-Pentachlorobiphenyl)	38380-02-8	Soil	0.15	0.50	0.088	0.5	ug/kg	39-118	20-137	40
8082 Congeners	PCB90 (2,2',3,4',5-Pentachlorobiphenyl)	68194-07-0	Soil	0.094	0.50	0.1	0.5	ug/kg	45-191	45-191	40
8082 Congeners	PCB95 (2,2',3,5',6-Pentachlorobiphenyl)	38379-99-6	Soil	0.049	0.50	0.1	0.5	ug/kg	70-130	70-130	40
8082 Congeners	PCB97 (2,2',3',4,5-Pentachlorobiphenyl)	41464-51-1	Soil	0.09	0.50	0.175	0.5	ug/kg	70-130	70-130	40
8082 Congeners	PCB99 (2,2',4,4',5-Pentachlorobiphenyl)	38380-01-7	Soil	0.079	0.50	0.175	0.5	ug/kg	70-130	70-130	40
8082 Congeners	Tetrachloro-m-xylene (Surr.)	877-09-8	Soil	NA	NA	NA	NA	%	10-135	NA	NA

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CAS/KELSO DATA QUALITY OBJECTIVES cont.

METHOD	ANALYTE	CAS No.	MATRIX	MDLa	MRL	LODb	LOQc	UNITS	Accuracy (LCS %Rec.)	Matrix Spike (%Rec.)	Precision (% RPD)
8082 Congeners	Tetrachloro-m-xylene (Surr.)	877-09-8	Tissue	NA	NA	NA	NA	%	27-122	NA	NA
8082 Congeners	PCB 1	2051-60-7	Tissue	1.5	5.0	3.25	5	ug/Kg	70-130	70-130	40
8082 Congeners	PCB 101	37680-73-2	Tissue	0.39	0.50	0.275	0.5	ug/Kg	70-130	70-130	40
8082 Congeners	PCB 105	32598-14-4	Tissue	0.10	0.50	0.325	0.5	ug/Kg	26-123	30-118	40
8082 Congeners	PCB 110	38380-03-9	Tissue	0.34	0.50	0.275	0.5	ug/Kg	70-130	70-130	40
8082 Congeners	PCB 114	74472-37-0	Tissue	0.089	0.50	0.275	0.5	ug/Kg	30-122	53-105	40
8082 Congeners	PCB 118	31508-00-6	Tissue	0.11	0.50	0.325	0.5	ug/Kg	16-149	19-140	40
8082 Congeners	PCB 119	56558-17-9	Tissue	0.12	0.50	0.325	0.5	ug/Kg	70-130	70-130	40
8082 Congeners	PCB 123	65510-44-3	Tissue	0.083	0.50	0.275	0.5	ug/Kg	29-128	55-106	40
8082 Congeners	PCB 126	57465-28-8	Tissue	0.14	0.50	0.325	0.5	ug/Kg	24-131	25-128	40
8082 Congeners	PCB 128	38380-07-3	Tissue	0.16	0.50	0.325	0.5	ug/Kg	15-127	26-113	40
8082 Congeners	PCB 132	38380-05-1	Tissue	0.20	0.50	0.275	0.5	ug/Kg	70-130	70-130	40
8082 Congeners	PCB 138	35065-28-2	Tissue	0.091	0.50	0.275	0.5	ug/Kg	36-120	40-110	40
8082 Congeners	PCB 141	52712-04-6	Tissue	0.50	0.50	0.275	0.5	ug/Kg	70-130	70-130	40
8082 Congeners	PCB 149	38380-04-0	Tissue	0.12	0.50	0.325	0.5	ug/Kg	70-130	70-130	40
8082 Congeners	PCB 151	52663-63-5	Tissue	0.089	0.50	0.275	0.5	ug/Kg	70-130	70-130	40
8082 Congeners	PCB 153	35065-27-1	Tissue	0.13	0.50	0.325	0.5	ug/Kg	22-123	25-111	40
8082 Congeners	PCB 156	38380-08-4	Tissue	0.56	1.0	0.275	0.5	ug/Kg	39-124	52-106	40
8082 Congeners	PCB 157	69782-90-7	Tissue	0.21	0.50	0.325	0.5	ug/Kg	24-123	25-132	40
8082 Congeners	PCB 158	74472-42-7	Tissue	0.096	0.50	0.325	0.5	ug/Kg	22-120	43-103	40
8082 Congeners	PCB 166	41411-63-6	Tissue	0.12	0.50	0.325	0.5	ug/Kg	70-130	70-130	40
8082 Congeners	PCB 167	52663-72-6	Tissue	0.50	0.50	0.275	0.5	ug/Kg	10-155	10-155	40
8082 Congeners	PCB 168	59291-65-5	Tissue	0.10	0.50	0.325	0.5	ug/Kg	70-130	70-130	40
8082 Congeners	PCB 169	32774-16-6	Tissue	0.089	0.50	0.325	0.5	ug/Kg	15-130	10-131	40
8082 Congeners	PCB 170	35065-30-6	Tissue	0.38	0.50	0.325	0.5	ug/Kg	20-115	10-145	40
8082 Congeners	PCB 174	38411-25-5	Tissue	0.13	0.50	0.325	0.5	ug/Kg	70-130	70-130	40
8082 Congeners	PCB 177	52663-70-4	Tissue	0.38	0.50	0.275	0.5	ug/Kg	70-130	70-130	40
8082 Congeners	PCB 18	37680-65-2	Tissue	0.098	0.50	0.275	0.5	ug/Kg	40-114	41-117	40
8082 Congeners	PCB 180	35065-29-3	Tissue	0.34	0.50	0.275	0.5	ug/Kg	41-124	48-119	40

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8082 Congeners	PCB 183	52663-69-1	Tissue	0.15	0.50	0.275	0.5	ug/Kg	23-136	38-122	40
8082 Congeners	PCB 184	74472-48-3	Tissue	0.13	0.50	0.275	0.5	ug/Kg	33-118	51-102	40
8082 Congeners	PCB 187	52663-68-0	Tissue	0.083	0.50	0.275	0.5	ug/Kg	37-129	22-141	40
8082 Congeners	PCB 189	39635-31-9	Tissue	0.18	0.50	0.275	0.5	ug/Kg	32-117	51-106	40
8082 Congeners	PCB 194	35694-08-7	Tissue	0.070	0.50	0.275	0.5	ug/Kg	70-130	70-130	40
8082 Congeners	PCB 195	52663-78-2	Tissue	0.33	0.50	0.275	0.5	ug/Kg	38-112	43-108	40
8082 Congeners	PCB 201	40186-71-8	Tissue	0.14	0.50	0.275	0.5	ug/Kg	70-130	70-130	40
8082 Congeners	PCB 203	52663-76-0	Tissue	0.29	0.50	0.275	0.5	ug/Kg	70-130	70-130	40
8082 Congeners	PCB 206	40186-72-9	Tissue	0.20	0.50	0.275	0.5	ug/Kg	39-116	44-109	40
8082 Congeners	PCB 209	2051-24-3	Tissue	0.15	0.50	0.275	0.5	ug/Kg	36-122	39-118	40
8082 Congeners	PCB 28	7012-37-5	Tissue	0.13	0.50	0.275	0.5	ug/Kg	44-137	41-146	40
8082 Congeners	PCB 31	16606-02-3	Tissue	0.11	0.50	0.325	0.5	ug/Kg	70-130	70-130	40
8082 Congeners	PCB 33	38444-86-9	Tissue	0.50	0.50	0.275	0.5	ug/Kg	70-130	70-130	40
8082 Congeners	PCB 37	38444-90-5	Tissue	0.10	0.50	0.325	0.5	ug/Kg	70-130	70-130	40
8082 Congeners	PCB 44	41464-39-5	Tissue	0.35	0.50	0.275	0.5	ug/Kg	43-127	40-133	40
8082 Congeners	PCB 49	41464-40-8	Tissue	0.11	0.50	0.325	0.5	ug/Kg	70-130	70-130	40
8082 Congeners	PCB 5	16605-91-7	Tissue	0.10	0.50	0.325	0.5	ug/Kg	70-130	70-130	40
8082 Congeners	PCB 52	35693-99-3	Tissue	0.39	0.50	0.275	0.5	ug/Kg	39-119	29-132	40
8082 Congeners	PCB 56	41464-43-1	Tissue	0.36	0.50	0.275	0.5	ug/Kg	70-130	70-130	40
8082 Congeners	PCB 60	33025-41-1	Tissue	0.097	0.50	0.325	0.5	ug/Kg	27-186	16-172	40
8082 Congeners	PCB 66	32598-10-0	Tissue	0.59	1.0	0.275	0.5	ug/Kg	35-125	31-134	40
8082 Congeners	PCB 70	32598-11-1	Tissue	0.28	0.50	0.275	0.5	ug/Kg	70-130	70-130	40
8082 Congeners	PCB 74	32690-93-0	Tissue	0.29	0.50	0.275	0.5	ug/Kg	70-130	70-130	40
8082 Congeners	PCB 77	32598-13-3	Tissue	0.12	0.50	0.325	0.5	ug/Kg	21-129	11-139	40
8082 Congeners	PCB 8	34883-43-7	Tissue	0.10	0.50	0.275	0.5	ug/Kg	47-124	54-121	40
8082 Congeners	PCB 81	70362-50-4	Tissue	0.16	0.50	0.325	0.5	ug/Kg	70-130	70-130	40
8082 Congeners	PCB 87	38380-02-8	Tissue	0.16	0.50	0.275	0.5	ug/Kg	30-119	36-113	40
8082 Congeners	PCB 90	68194-07-0	Tissue	0.11	0.50	0.325	0.5	ug/Kg	70-130	70-130	40
8082 Congeners	PCB 90 + PCB 101	CASID30302	Tissue	1.0	1.0		0.5	ug/Kg	70-130	70-130	40
8082 Congeners	PCB 95	38379-99-6	Tissue	0.15	0.50	0.325	0.5	ug/Kg	70-130	70-130	40
8082 Congeners	PCB 97	41464-51-1	Tissue	0.18	0.50	0.275	0.5	ug/Kg	70-130	70-130	40
8082 Congeners	PCB 99	38380-01-7	Tissue	0.14	0.50	0.275	0.5	ug/Kg	70-130	70-130	40
8082 Congeners	Tetrachloro-m-xylene (Surr.)	877-09-8	Tissue	NA	NA			%	27-122	-	-

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a Method Detection Limits are subject to change as new MDL studies are completed.
a MDL is the smallest analyte concentration that can be demonstrated to be different from zero with 99% confidence
b The LOD is the smallest amount of a substance that must be present in a sample in order to be detected with 99% confidence.

Verification is acceptable if the response is > 3x instrument noise & 2nd column confirmed or pattern.

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STANDARD OPERATING PROCEDURE

AUTOMATED SOXHLET EXTRACTION

EXT-3541

Revision 7

Effective date: January 27, 2012

Approved By:

Elissa Erickson — Supervisor — 12-27-11 — Date

Julie Nash — QA Manager — 12-27-11 — Date

Laboratory Manager — 12/27/11 — Date

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AUTOMATED SOXHLET EXTRACTION

1. SCOPE AND APPLICATION

1.1. This procedure uses techniques described in EPA Method 3541 for extracting nonvolatile and semi-volatile organic compounds from solids such as soils, sediments, sludges, wastes, and tissues.

1.2. This method is applicable to the isolation and concentration of water insoluble and slightly water soluble organics in preparation for a variety of chromatographic procedures.

2. SUMMARY OF METHOD

2.1. This procedure describes the automated Soxhlet extraction process. The Soxhlet extraction ensures intimate contact of the sample matrix with the extraction solvent over a period of time. The solid sample is mixed with anhydrous sodium sulfate, placed in an extraction thimble above a plug of glass wool, and extracted using an appropriate solvent on a Soxtherm extractor for a prescribed amount of time.

2.2. Following the extraction period, the resulting extract is then dried if necessary, concentrated, and as necessary, exchanged into a solvent compatible with the cleanup or determinative step being employed.

3. DEFINITIONS

3.1. **Batch** - A batch of samples is a group of environmental samples that are prepared and/or analyzed together as a unit with the same process and personnel using the same lot(s) of reagents. It is the basic unit for analytical quality control.

3.1.1. Preparation Batch - A preparation batch is composed of one to twenty field samples, all of the same matrix, meeting the criteria in Section 3.3 and with a maximum time between the start of processing of the first and last samples in the batch to be 24 hours.

3.2. **Sample**

3.2.1. Field Sample - An environmental sample collected and delivered to the laboratory for analysis; a.k.a., client's sample.

3.2.2. Laboratory Sample - A representative portion, aliquot, or subsample of a field sample upon which laboratory analyses are made and results generated.

3.3. **Quality System Matrix** - The *matrix* of an environmental sample is distinguished by its physical and/or chemical state and by the program for which the results are intended. The

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following sections describe the matrix distinctions. These matrices shall be used for purpose of batch and quality control requirements.

3.3.1. Animal tissue - Any tissue sample of an animal, invertebrate, marine organism, or other origin; such as fish tissue/organs, shellfish, worms, or animal material.

3.3.2. Solids - Any solid sample such as soil, sediment, sludge, and other materials with >15% settleable solids.

3.3.3. Chemical waste - Any sample of a product or by-product of an industrial process that results in a matrix not described in one of the matrices in Sections 3.3.1 through 3.3.6. These can be such matrices as non-aqueous liquids, solvents, oil, etc.

3.3.4. Miscellaneous matrices – Samples of any composition not listed in 3.3.1 – 3.3.4. These can be such matrices as plant material, paper/paperboard, wood, auto fluff, mechanical parts, filters, wipes, etc. Such samples shall be batched/grouped according to their specific matrix.

3.4. Matrix Spike/Duplicate Matrix Spike (MS/DMS) Analysis - In the matrix spike analysis, predetermined quantities of target analytes are added to a sample matrix prior to sample preparation and analysis. The purpose of the matrix spike is to evaluate the effects of the sample matrix on the method used for the analysis. Duplicate samples are spiked, and analyzed as a MS/DMS pair. Percent recoveries are calculated for each of the analytes detected. The relative percent difference (RPD) between the duplicate spikes (or samples) is calculated and used to assess analytical precision. The concentration of the spike should be at the mid-point of the calibration range or at levels specified by a project analysis plan.

3.5. Laboratory Duplicates (DUP) – Duplicates are additional replicates of samples that are subjected to the same preparation and analytical scheme as the original sample. The relative percent difference (RPD) between the sample and its duplicate is calculated and used to assess analytical precision.

3.6. Surrogate - Surrogates are organic compounds which are similar to analytes of interest in chemical composition, extraction and chromatography, but which are not normally found in environmental samples. The purpose of the surrogates is to evaluate the preparation and analysis of samples. These compounds are spiked into all blanks, standards, samples and spiked samples prior to extraction and analysis. Percent recoveries are calculated for each surrogate.

3.7. Method Blank (MB) - The method blank is an artificial sample composed of analyte-free water or solid matrix and is designed to monitor the introduction of artifacts into the analytical process. The method blank is carried through the entire analytical procedure.

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3.8. Laboratory Control Samples (LCS) – The LCS is an aliquot of analyte free water or analyte free solid to which known amounts target analytes are added. The LCS is prepared and analyzed in exactly the same manner as the samples. The percent recovery is compared to established limits and assists in determining whether the batch is in control.

4. INTERFERENCES

4.1. Phthalate esters can pose difficulties when performing sample extractions for organochlorine pesticides, PCBs, and other semi-volatile organics. Phthalates are easily extracted or leached from materials containing plastics during laboratory operations. Interferences from phthalates can best be minimized by avoiding contact with any plastic materials.

4.2. Contamination by carryover can occur whenever high-concentration and low-concentration samples are sequentially analyzed. All apparatus must be cleaned prior to use on individual samples.

4.3. Soap residue, which results in a basic pH on glassware surfaces, may cause degradation of certain analytes. Specifically, Aldrin, Heptachlor, and most OP pesticides will degrade in this situation. All glassware must be rinsed very carefully to avoid this problem.

4.4. Refer to SW-846 Method 3500 for additional discussion of interferences. Additional cleanup procedures are described in the applicable CAS SOP.

5. SAFETY

5.1. All appropriate safety precautions for handling solvents, reagents, and samples must be taken when performing this procedure. This includes the use of protective equipment (safety glasses, lab coats, gloves, etc.) and use of correct glassware handling practices.

5.2. Chemicals, reagents, standards, and samples must be handled as described in CAS safety policies, approved methods, and in MSDSs where available. Refer to the specific analytical method and the CAS Safety Manual for guidance.

6. RESPONSIBILITIES

6.1. It is the responsibility of the analyst to perform the analysis according to this SOP and to complete all documentation required for data review. Analysis and interpretation of the results are performed by personnel in the laboratory who have demonstrated the ability to generate acceptable results utilizing this SOP. This demonstration is in accordance with the training program of the laboratory. Final review and sign-off of the data is performed by the department supervisor/manager or designee.

6.2. It is the responsibility of the department supervisor/manager to document analyst training. Documenting method proficiency, as described in the determinative method, is also the responsibility of the department supervisor/manager.

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7. APPARATUS AND MATERIALS

7.1. Soxtherm automated extraction system

7.1.1. Extraction unit controller

7.1.2. Beakers, 54 x 130mm

7.1.3. Thimbles, 33mm inner diameter, glass or cellulose

7.1.4. Metal thimble holder

7.2. Chiller unit

7.3. Boiling chips – Teflon, pre-cleaned by rinsing or extraction.

7.4. Water bath - Heated, with concentric ring cover, capable of temperature control (± 5°C). The bath should be used in a hood.

7.5. Vials - Glass, 2 ml capacity, with Teflon lined screw or crimp top.

7.6. Glass wool - contaminant free.

7.7. Disposable glass Pasteur pipet and bulb.

7.8. Apparatus for grinding.

7.9. Analytical balance - 0.01 g.

7.10. Test tubes

7.11. N-Evap concentrator with nitrogen source

7.12. Graduated pipets, 0.5, 1, 2 and 5 mL. Pipets are pre-tested by lot for accuracy.

8. REAGENTS

8.1. Pesticide grade chemicals shall be used in all tests. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lowering the accuracy of the determination. The preparation for all laboratory prepared reagents and solutions must be documented in a laboratory logbook. Refer to the SOP *Reagent/Standards Login and Tracking (ADM-RTL)* for the complete procedure and documentation requirements.

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8.2. Organic-free reagent water. All references to water in this method refer to organic-free reagent water, as defined in Chapter One of SW-846.

8.3. Sodium sulfate (granular or powder, anhydrous), Na_2SO_4. Purify by heating at 400°C for 4 hours.

8.4. Matrix sand. Purify by heating at 400°C for 4 hours.

8.5. Corn oil

8.6. Blank tissue matrix. Analyte free ground turkey or equivalent

8.7. Extraction Solvents - Samples are extracted using one of the following solvent systems:

8.7.1. Acetone/Hexane (1:1) (v/v), CH_3COCH_3/C_6H_{14}. Pesticide quality or equivalent. Soil, sediment, and aqueous sludge samples.

8.7.2. Dichloromethane/Acetone (1:1 v/v), CH_2Cl_2/CH_3COCH_3. Pesticide quality or equivalent. Soil, sediment, and aqueous sludge samples.

8.7.3. Dichloromethane (DCM), CH_2Cl_2. Pesticide quality or equivalent. Other miscellaneous sample matrices.

9. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

9.1. Refer to the applicable section in the determinative SOP for sample collection, preservation, and holding times.

9.2. The extract holding time is 40 days from sample preparation to analysis.

10. PREVENTIVE MAINTENANCE

10.1. Routine cleaning of the extraction apparatus is necessary, including all parts exposed to contact with samples, especially extraction thimbles and the Soxtherm apparatus.

10.2. The operating temperature of the soxtherm is monitored every quarter (March, June, September, and December) with a NIST traceable digital thermometer and probe. The soxtherm temperature is set at the controller to heat up to the extraction temperature of 140º C. Once the control box reads 140º C the probe is placed directly on each individual heating surface and the temperature is recorded in the soxtherm maintenance logbook (SVMP-SOX-01).

10.3. The extraction time on the controller is verified quarterly by recording the start and stop times of an extraction and recording in the soxtherm maintenance logbook.

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11. RESPONSIBILITIES

It is the responsibility of the analyst to perform the analysis according to this SOP and to complete all documentation required for data review. Analysis and interpretation of the results are performed by personnel in the laboratory who have demonstrated the ability to generate acceptable results utilizing this SOP. This demonstration is in accordance with the training program of the laboratory. Final review and sign-off of the data is performed by the department supervisor/manager or designee.

12. PROCEDURE

12.1. Record all extraction and sample information on the applicable benchsheet. To assist the analyst, a brief description of the procedure is given on the backside of the benchsheet. See Attachment A.

12.2. Sample Handling

12.2.1. Sediment/soil samples - Mix the sample thoroughly, especially composited samples. Discard any foreign objects such as sticks, leaves, and rocks.

12.2.2. Waste samples - Samples consisting of multi phases have each phase extracted separately. If sample contains a significant portion of water, water is pulled off and extracted. Oil phases are pulled off, usually diluted in hexane or iso-octane and extracted. This procedure is for solids only.

12.2.3. Dry waste samples amenable to grinding - Grind or otherwise subdivide the waste so that it passes through a 1 mm sieve.

12.2.4. Gummy, fibrous, or oily materials not amenable to grinding should be cut, shredded, or otherwise broken up to allow mixing, and maximum exposure of the sample surfaces for extraction. The professional judgment of the analyst is required for handling these difficult matrices.

12.2.5. Tissue samples need to be ground or cut to allow mixing and improve exposure of the sample to the solvent.

12.3. Determinations of sample % dry weight - In certain cases, sample results are desired based on dry weight basis. Refer to the *SOP for Total Solids* GEN-160.3). If the determination is performed by the organics preparation personnel, a portion of the sample for this determination should be weighed out at the same time as the portion used for analytical determination.

12.4. Sample extraction and extract concentration

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12.4.1. Pre-rinse glassware (beaker, thimble with glass wool, and metal clip) before use. To clean the glassware place 50mls of dichloromethane in beaker and run a short extraction program. Example 10 min. boil time, 1 reduction A, 10 min. extraction, 1 reduction B, 30 min. solvent cooling, temp. 140º C.

12.4.2. Refer to the determinative SOP (see Table 1 for a list of applicable SOPs) for the preparation, concentration, storage, and expiration for the surrogate, LCS, and MS spiking solutions. These SOPs also list the resulting final spike concentrations.

12.4.3. Weigh out the specified amount (see test-specific benchsheets attached) of the solid sample to be extracted. Be careful not to weigh out more sample than what can fit into a maximum of 2 thimbles.

12.4.4. Blend the specified sample amount with as little sodium sulfate as possible, so as not to overload the extraction thimble, but still achieve sample drying. The type of sodium sulfate used is dependent upon the method. See Table 2 for further details on which type of sulfate to use.

12.4.5. The composition of the MB and LCS is prepared is dependent upon the method. See Table 2 for further details.

12.4.6. The MB and LCS is handled in the same manner as the client samples (i.e. drying in hood or any other temporary storage location).

12.4.7. Transfer the dry sample into the thimble that contains a plug of glass wool to prevent the sample from dropping into the beaker. Use a second thimble to prevent overfilling or over packing the thimble.

12.4.8. Rinse each sample beaker with a small amount of dichloromethane and transfer rinsate to thimble. Perform rinse three times to ensure quantitative transfer.

12.4.9. Add the surrogate standard spiking solution onto the samples. For the sample(s) in each analytical batch selected for QC spiking (LCS and MS samples), add the appropriate volume of the appropriate spiking standard. Each standard should be brought to room temperature before using. Addition of surrogate and spike is routinely witnessed by a second analyst to assure completeness. Also, the witness should immediately follow the spiking of each sample with the addition of a small amount of dichloromethane.

12.4.10. Add enough dichloromethane or appropriate extraction solvent to cover each sample. This will allow sample to remain covered by solvent during the entire boiling step. If the samples do not equally fill the thimbles feel free to mix and match sample and dichloromethane amounts so that all samples on a single extraction unit have about the same solvent level. Each unit can run a separate

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program, but a single unit does not allow multiple programs. In the program this step is referred to as boiling time.

12.4.11. The boiling step is set to be an hour. During this time the solvent will boil through and over the sample. The majority of the extraction will occur in this step. The temperature of the hot plates should be set for 140°C for dichloromethane and DCM/ACE. When the boiling time is completed the soxtherm needs to be programmed for the proper number of solvent reductions. Solvent reductions happen in 15mL increments as the calibrated cup is filled by the condensing solvent. When programming the number of reductions the original amount of solvent needs to be taken into consideration. The aim of this step is to bring the solvent level below the tip of the thimble with 50mls of being the desired amount. In the soxtherm program this is referred to as solvent reduction A.

12.4.12. Once the solvent level is below the thimble the extraction time can start. This lasts 1 hour. During this step the solvent drips through the sample to rinse through any remaining analytes. When this is done reduction B can begin. This step should take the solvent level to the desired amount of $\geq$ 10 mL. Care should be taken during this step, since each beaker seals differently. Some beakers seal very well and others allow more solvent losses. Also all samples are not created equal. Some samples will trap more solvent than others.

12.4.13. If the solvent level has reached the desired stopping point before the programmed numbers of reductions are completed, remove the beaker and immediately cover with foil until cooled. Conversely, if the solvent level of the samples on an instrument is higher than desired, additional solvent reductions can be programmed. Once the desired solvent amount has been reached, remove the beaker and immediately cover with foil.

12.4.14. Nitrogen blowdown is used to further concentrate the extract and to adjust the extract to the final volume. If solvent exchange is required (e.g. GC-ECD analyses) it is done at this point.

12.4.14.1. Using the N-Evap, place the concentrator tube in a warm water bath (approximately 35°C) and evaporate the solvent volume to the required level using a gentle stream of clean, dry nitrogen (filtered through a column of activated carbon).

CAUTION: Do not use plasticized tubing between the carbon trap and the sample.

12.4.14.2. The internal wall of the tube must be rinsed down several times with the appropriate final solvent during the operation. During evaporation, the solvent level in the tube must be positioned to prevent water from condensing into the sample (i.e., the solvent level should be below the level

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of the water bath). The volume of extract in the tube must be monitored during blowdown to avoid loss of more volatile analytes. Under normal operating conditions, the extract should not be allowed to become dry.

CAUTION: When the volume of solvent is reduced below 1 ml, semivolatile analytes may be lost.

12.4.14.3. Adjust the extract final volume to the prescribed volume with the solvent last used. Measure the final extract volume using a 0.5, 1, or 2 mL graduated pipet, depending on the test.

12.4.15. If extract cleanup is to be performed, concentrate the extract to the appropriate volume. Proceed to the applicable cleanup procedure and SOP.

12.4.16. During the final volume step, transfer the extracts to an appropriate storage or autosampler vial. Label the vial with the sample or QC identification and store in the extract storage area. The extracts obtained may now be analyzed for the target analytes using the appropriate determinative technique. The extract holding time is 40 days from sample preparation to analysis.

13. QUALITY CONTROL

13.1. Refer to the SOP for the determinative method and *SOP for Sample Batches* for minimum QC requirements. Project-specific batching protocols may also be required.

13.2. The QC solutions required by the method must be added as described in the analytical method. The amount and identification of QC solutions added must be documented on the bench sheet. Any reagent blanks, laboratory control samples, or matrix spike samples should be subjected to exactly the same analytical procedures as those used on actual samples.

14. DATA REPORTING AND REVIEW

14.1. Preparation of all samples must be documented on a bench sheet. All information regarding the sample(s) extracted, aliquoting, QC spiking, extraction steps, etc. must be documented by the person(s) performing the extraction.

14.2. The bench sheet must be reviewed by the extraction lead, supervisor, or instrument lab analyst. The instrument lab analyst should sign-off on the bench sheet, thus accepting custody of the extracts.

15. CONTINGENCIES FOR HANDLING OUT-OF-CONTROL OR UNACCEPTABLE DATA

15.1. Refer to the SOP for *Corrective Action (ADM-CA)* for procedures for corrective action. Personnel at all levels and positions in the laboratory are to be alert to identifying problems and nonconformities when errors, deficiencies, or out-of-control situations are detected.

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15.2. Handling out-of-control or unacceptable data

15.2.1. On-the-spot corrective actions that are routinely made by analysts and result in acceptable analyses should be documented as normal operating procedures, and no specific documentation need be made other than notations in laboratory maintenance logbooks, runlogs, for example.

15.2.2. Documentation of a nonconformity must be done using a Nonconformity and Corrective Action Report (NCAR) when:

- Corrective action is not taken or not possible
- Corrective action fails to correct an out-of-control problem on a laboratory QC or calibration analysis.
- Reanalysis corrects the nonconformity but is not a procedurally compliant analysis.

16. METHOD PERFORMANCE

Available method performance data is given in the reference method. In addition, this procedure was validated through single laboratory studies of accuracy and precision as specified in the determinative procedures.

17. POLLUTION PREVENTION

It is the laboratory's practice to minimize the amount of solvents and reagents used to perform this method wherever technically sound, feasibly possible, and within method requirements. Standards are prepared in volumes consistent with laboratory use in order to minimize the volume of expired standards to be disposed of. The threat to the environment from solvents and/or reagents used in this method may be minimized when recycled or disposed of properly.

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18. WASTE MANAGEMENT

18.1. The laboratory will comply with all Federal, State and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions as specified in the CAS EH&S Manual.

18.2. This method uses Dichloromethane and any waste generated from this solvent must be placed in the collection cans in the lab. The solvent will then be added to the hazardous waste storage area and recycled off site.

18.3. This method uses non-halogenated solvents and any waste generated from this solvent must be placed in the collection cans in the lab. The solvent will then be added to the hazardous waste storage area and disposed of in accordance with Federal and State regulations.

18.4. All extracted soil samples are collected in a labeled waste container for disposal in accordance with applicable state and federal regulations governing waste management.

19. TRAINING

19.1. Training outline

19.1.1. Review literature (see references section). Read and understand the SOP. Also review the applicable MSDS for all reagents and standards used. Following these reviews, observe the procedure as performed by an experienced analyst at least three times.

19.1.2. The next training step is to assist in the procedure under the guidance of an experienced analyst. During this period, the analyst is expected to transition from a role of assisting, to performing the procedure with minimal oversight from an experienced analyst.

19.1.3. Perform an initial precision and recovery (IPR) study as described above for solid samples. Summaries of the IPR are reviewed and signed by the supervisor. Copies may be forwarded to the employee's training file.

19.2. Training is documented following the SOP *for Documentation of Training*.

NOTE: When the analyst training is documented by the supervisor on internal training documentation forms, the supervisor is acknowledging that the analyst has read and understands this SOP and that adequate training has been given to the analyst to competently perform the analysis independently.

20. METHOD MODIFICATIONS

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20.1. Reference method 3541 uses 1:1 Acetone:Hexane for the extraction of 8081 and 8082 analytes. This procedure uses 100% Methylene chloride

20.2. Reference method 3541 uses 1:1 Acetone:Methylene chloride for the extraction of semi-volatile organics. This procedure uses 100% Methylene chloride

21. REFERENCES

21.1. EPA SW-846, Test Methods For Evaluating Solid Waste, Third Edition, Update II, September 1994, Method 3541, Revision 0.

21.2. EPA SW-846, Test Methods For Evaluating Solid Waste, Third Edition, Update IV, February 2007, Method 3500C, Revision 3

22. CHANGES SINCE THE LAST REVISION

22.1. Definitions section 3 is new
22.2. Sec 7 added powder sulfate, matrix sand, corn oil, blank tissue matrix
22.3. Sec 8.1 updated language
22.4. Sec 12.3 updated sop reference
22.5. Sec 12.4.4 – 12.4.7 re-written for clarity and added quantitative transfer directions
22.6. Sec 12.4.11 changed 5-15mL to >10mL
22.7. Sec 18.4 is new
22.8. Sec 21.2 – updated reference
22.9. Added new table 2

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TABLE 1

APPLICABLE DETERMINATIVE SOPs

POLYNUCLEAR AROMATIC HYDROCARBONS BY GAS CHROMATOGRAPHY/MASS SPECTROMETRY SIM	SVM-8270P
SEMIVOLATILE ORGANIC COMPOUNDS BY GC/MS	SVM-8270D
SEMIVOLATILE ORGANIC COMPOUNDS BY GC/MS – LOW LEVEL PROCEDURE	SVM-8270L
SEMIVOLATILE ORGANIC COMPOUNDS BY GC/MS SELECTED ION MONITORING	SVM-8270S
ORGANOCHLORINE PESTICIDES BY GAS CHROMATOGRAPHY: CAPILLARY COLUMN TECHNIQUE	SOC-8081
PCBS AS AROCLORS	SOC-8082Ar
CONGENER SPECIFIC DETERMINATION OF PCB BY GC/ECD	SOC-8082Co
CHLORINATED PHENOLICS BY IN-SITU ACETYLATION AND GC/MS	SVM-1653A
DETERMINATION OF NITROGEN OR PHOSPHORUS CONTAINING PESTICIDES	SOC-8141
NONYLPHENOLS ISOMERS AND NONYLPHENOL ETHOXYLATES	SVM-NONLY
CHLORINATED PESTICIDES BY GC/MS/MS, EPA METHOD 1699 MODIFIED	SVM-PESTMS2

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TABLE 2
Soxtherm Method Aid

Method	Matrix	Target amount	MB	LCS
8081	Tissue	20g	Granular Sulfate	Blank Tissue + Granular Sulfate
8082	Tissue	20g	Granular Sulfate	Blank Tissue + Granular Sulfate
8082-Con	Tissue	20g	Granular Sulfate	Blank Tissue + Granular Sulfate
8081	Soil	10g	Powder Sulfate*	Matrix sand + Powder Sulfate*
8082	Soil	10g	Powder Sulfate*	Matrix sand + Powder Sulfate*
8081-L	Soil	20g	Powder Sulfate*	Matrix sand + Powder Sulfate*
8082-L	Soil	20g	Powder Sulfate*	Matrix sand + Powder Sulfate*
8082-Con	Soil	20g	Powder Sulfate*	Matrix sand + Powder Sulfate*
8015	Soil	20g	Powder Sulfate*	Matrix sand + Powder Sulfate*
8015 co-extract	Soil	20g	Powder Sulfate*	Matrix sand + Powder Sulfate*
Lipids	Tissue	10g	Granular Sulfate	Blank Tissue + Granular Sulfate
8270 Pest OP	Soil	10g	Granular Sulfate	Granular Sulfate
8270 Pest OP	Tissue	5g	Granular Sulfate	Granular Sulfate
8270 OC Pest	Soil	10g	Granular Sulfate	Granular Sulfate
8270 OC Pest	Tissue	2g	Granular Sulfate	Granular Sulfate
PAH ALK	Soil	10g	Granular Sulfate	Granular Sulfate
PAH ALK co-extract	Soil	20g	Granular Sulfate	Granular Sulfate

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TABLE 2-cont.

Method	Matrix	Target amount	MB	LCS
PAH ALK	Tissue	10g	Granular Sulfate + Corn oil	Granular Sulfate + Oil
SIM PAH	Soil/ Paperboard	10g	Granular Sulfate	Granular Sulfate
SIM PAH ULL	Solids	20g	Granular Sulfate	Granular Sulfate
SIM PAH	Tissue	10g	Granular Sulfate + Corn oil	Granular Sulfate + Oil
SIM PAH ULL	Tissue	10g	Granular Sulfate + Corn oil	Granular Sulfate + Oil
SIM PAH PCP	Soil/Tissue	10g	Granular Sulfate	Granular Sulfate
Nonylphenols	Solids	10g	Granular Sulfate	Granular Sulfate
Nonylphenols	Paperboard	1g	Granular Sulfate	Granular Sulfate
PBDE/PBB	Soil/Tissue	10g	Granular Sulfate	Granular Sulfate
PBDE/PBB	Paperboard	1g	Granular Sulfate	Granular Sulfate
8270	Soil/ Paperboard	20g	Granular Sulfate	Granular Sulfate
8270 LL	Soil	30g	Granular Sulfate	Granular Sulfate
8270 SIM	Tissue	5g	Granular Sulfate	Granular Sulfate

* If sample has high water content a mixture of Granular and Powder sulfate can be used. The same mixture is then used in the MB and LCS.

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Attachments

Test-Specific Bench Sheets

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Additional Prep Information For Hydrocarbon in Soils by EPA 3541

Service Request #_____ **Work Group #** _____

Solvents/Reagents used:

Date/Time/Initials Weighed:_____ **Balance ID:**_____ **Calibration Verified** ☐

Storage Location (if not extracted same day):_____

DCM Lot #_____**Sulfate Lot #**_____**Matrix Sand Lot #**_____

Soxtherm Start (Time/Date/Initial):_____

Soxtherm Stop (Time/Date/Initial):_____

Cleanups: (Check paperwork to see if cleanups are necessary)

N-Evap Date/Time/Initial:_____ **Temp/Thermometer ID:**_____

Sulfuric Acid Clean-up (3665) (Date/Time/Initials):_____ **Acid Lot #**_____

Silica Gel Clean-up (3630) (Date/Time/Initials):_____ **Silica Gel Lot #**_____

Archive:

Vial:_____ **Vial Storage:**_____

Archived Extract Storage:_____

Comments/Observations:_____

_____

_____

_____

_____

Bench Sheet Review Check List
☐ Hold Times Met (if no, Reason: _____)
☐ Prep date, dept, method, product code correct in stealth
☐ Spike Information correct
☐ Weights/Volumes and units correct on raw and final bench sheets
☐ Sample IDs have been checked—Bottle numbers appended if required
☐ Names present for: Started by, Completed by, relinquished by, and witnessed by.
☐ Training has been circled
☐ Extract Storage recorded
☐ Additional Prep Sheet completely filled out (NA or line out Blanks)
☐ All clean-ups have been noted on additional prep sheet
☐ Signed service request with Form V, if applicable, has been attached

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Procedure for extraction of Hydrocarbons by EPA Method 3541 (8015/NWTPH/AK102/103)

QC Requirements:

DX/HCID: DUP every 10 samples, LCS, MB (DLCS required if insufficient sample for DUPs)

8015B DRO: MS, DMS, LCS, MB

AK DRO: MS, DMS, LCS, DLCS, MB

Note: AKs must be extracted separately, cannot be batched with the tests above.

1. **Mix the sample thoroughly. Weigh appropriate amount into a beaker. Record the weight to the nearest 0.01 grams. Use muffled matrix sand for the LCS and muffled sodium sulfate for the Method Blank.**
2. **Mix the samples with a sufficient amount of muffled sodium sulfate to completely dry the sample.**
3. **Transfer samples to Soxtherm thimbles that have been cycled with DCM.**
4. **Add surrogates and spikes as necessary.**
5. **Add 150mL of DCM to the extraction beaker.**
6. **Extract using the extraction method.**
7. **Take extract to appropriate final volume in DCM.**
8. **Do acid silica gel cleanup (3665/3630) if requested.**
9. **Place extract into white autosampler vial for analysis.**
10. **Archive any remaining extract and record location on benchsheet.**

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Additional Prep Information For Pest/PCB Soils by EPA 3541

Service Request #_____ Work Group # _____

Solvents/Reagents used:

Date/Time/Initials Weighed:_____ Balance ID:_____ Calibration Verified ☐

Storage Location (if not extracted same day):_____

DCM Lot #_____Sulfate Lot #_____Matrix Sand Lot #_____

Soxtherm Start (Time/Date/Initial):_____

Soxtherm Stop (Time/Date/Initial):_____

Cleanups:

N-Evap Date/Time/Initial:_____ Temp/Thermometer ID:_____

GPC Clean-up (3640) (Time/Date/Initial):_____ (all samples)

Solvent Exchanged To Hexane (Time/Date/Initial):_____
S-Evap Date/Time/Initial:_____ Temp/Thermometer ID:_____

N-Evap Date/Time/Initial:_____ Temp/Thermometer ID:_____

Sulfuric Acid Clean-up (3665) (Time/Date/Initial):_____ Acid Lot #:_____

Clean-up #3:_____ ☐ all samples ☐ some samples:_____

Pest Vial:_____ Vial Storage:_____
PCB Vial:_____ Vial Storage:_____

Archived Extract Storage:_____

Comments/Observations:_____

_____

Bench Sheet Review Check List
❑ Hold Times Met (if no, Reason: _____)
❑ Prep date, dept, method, product code correct in stealth
❑ Spike Information correct
❑ Weights/Volumes and units correct on raw and final bench sheets
❑ Sample IDs have been checked—Bottle numbers appended if required
❑ Names present for: Started by, Completed by, relinquished by, and witnessed by.
❑ Training has been circled
❑ Extract Storage recorded
❑ Additional Prep Sheet completely filled out (NA or line out Blanks)
❑ All clean-ups have been noted on additional prep sheet
❑ Signed service request with Form V, if applicable, has been attached

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Procedure for extraction of Pesticides and PCB's by EPA Method 3541 (8081/8082)

1. Mix the sample thoroughly. Weigh appropriate amount into a beaker. Record the weight to the nearest 0.01 grams. Use muffled matrix sand for the LCS and muffled sodium sulfate for the Method Blank.
2. Mix the samples with a sufficient amount of muffled sodium sulfate to completely dry the sample.
3. Transfer samples to Soxtherm thimbles that have been cycled with DCM.
4. Add surrogates and spikes as necessary.
5. Add 150mL of DCM to the extraction beaker.
6. Extract using the extraction method.
7. Take extract to 10mL intermediate volume in DCM.
8. Do GPC cleanup (3640). Refer to CAS SOP for GPC clean-up.
9. Evaporate on the S-evap, keeping temperature between 70-75°C. Solvent exchange into Hexane when extracts reach approximately 10mL.
10. Concentrate extracts and do additional cleanups as necessary.
11. Place exactly 1mL of extract into a yellow vial for the pesticide analysis.
12. Perform a sulfuric acid cleanup (3665) on the remaining PCB extract according to CAS SOP.
13. Place PCB extract into green vial for analysis.

Test	Amount to Weigh (g)	Surr. Amount (uL)	Spike Amount (uL)	Final Volume (mL)
8081	10	500	250	10
8082	10	500	250	10
8081-L	20	200	100	4
8082-L	20	200	100	4
8082-CON	20	200 (Con)	200 (Con)	4

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Additional Prep Information For Pest/PCB Tissues by 3541

Service Request #_____ Work Group # _____

Solvents/Reagents used:

Date/Time/Initials Weighed:_____ Balance ID:_____ Calibration Verified ☐

Storage Location (if not extracted same day):_____

DCM Lot #_____Sulfate Lot #_____

Soxtherm Start (Time/Date/Initial):_____

Soxtherm Stop (Time/Date/Initial):_____

Cleanups:

N-Evap Date/Time/Initial:_____ Temp/Thermometer ID:_____

GPC Clean-up (3640) (Time/Date/Initial):_____ (all samples)

Solvent Exchanged To Hexane (Time/Date/Initial):_____ Hexane Lot #:_____

S-Evap Date/Time/Initial:_____ Temp/Thermometer ID:_____

N-Evap Date/Time/Initial:_____ Temp/Thermometer ID:_____

Florisil Clean-up (3620) (Time/Date/Initial): _____Florisil Lot #:_____

1:1 Hexane:Acetone Lot #:_____ 9:1 Hexane:Acetone Lot #:_____

N-Evap Date/Time/Initial:_____ Temp/Thermometer ID:_____

Sulfuric Acid Clean-up (3665) (Time/Date/Initial):_____Acid Lot #:_____

Archive:

Pest Vial:_____ Vial Storage:_____

PCB Vial:_____ Vial Storage:_____

Archived Extract Storage:_____

Comments/Observations:_____

_____

Bench Sheet Review Check List
☐ Hold Times Met (if no, Reason: _____)
☐ Prep date, dept, method, product code correct in stealth
☐ Spike Information correct
☐ Weights/Volumes and units correct on raw and final bench sheets
☐ Sample IDs have been checked—Bottle numbers appended if required
☐ Names present for: Started by, Completed by, relinquished by, and witnessed by.
☐ Training has been circled
☐ Extract Storage recorded
☐ Additional Prep Sheet completely filled out (NA or line out Blanks)
☐ All clean-ups have been noted on additional prep sheet
☐ Signed service request with Form V, if applicable, has been attached

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Procedure for extraction of Pesticides and PCB's in tissue by EPA Method 3541 (8081/8082)

1. Mix the sample thoroughly. Weigh appropriate amount into a beaker. Record the weight to the nearest 0.01 grams. Use ground turkey breast for the LCS and muffled sodium sulfate for the Method Blank.
2. Mix the samples with a sufficient amount of muffled sodium sulfate to completely dry the sample.
3. Transfer samples to Soxtherm thimbles that have been cycled with DCM.
4. Add surrogates and spikes as necessary.
5. Add 150mL of DCM to the extraction beaker.
6. Extract using the extraction method.
7. Take extract to 10mL intermediate volume in DCM.
8. Do GPC cleanup (3640). Refer to CAS SOP for GPC clean-up.
9. Evaporate on the S-evap, keeping temperature between 70-75°C. Solvent exchange into Hexane when extracts reach approximately 10mL.
10. Concentrate extracts and do a Florisil cleanup.
11. Take extracts to appropriate final volume.
12. Place exactly 1mL of extract into a yellow vial for the pesticide analysis.
13. Perform a sulfuric acid cleanup (3665) on the remaining PCB extract according to CAS SOP.
14. Place PCB extract into green vial for analysis.

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Additional Prep Information For EPA 3541

1653s by GCMS

Service Request _____ **Workgroup** _____

Sulfate Lot #: _____ **DCM (GC^2)Lot #:** _____

Date/Time/Initials Weighed:_____ **Balance ID:**_____ **Calibration Verified** ☐

Storage Location (if not extracted same day):_____

Glass Wool Lot#:_____

Soxtherm Start (Time/Date/Initial): _____

Soxtherm Stop (Time/Date/Initial): _____

Turbovap/ACN Solvent Exchange (Temp/ID/Time/Date/Initial): _____

K_2CO_3 Lot #_____ **Acetic Anhydride Lot #** _____

Hexane Lot #_____ **ACN Lot #** _____

Batch Start (Time/Date/Initial)_____

Batch Finish (Time/Date/Initial)_____

S-Evap (Temp/ID/Time/Date/Initial):_____

Turbo vap (Temp/ID/Time/Date/Initial):_____

Date Completed (Time/Date/Initial): _____

Comments/Observations:

_____

_____

_____

Bench Sheet Review Check List
☐ Hold Times Met (if no, Reason: _____)
☐ Prep date, dept, method, product code correct in stealth
☐ Spike Information correct
☐ Weights/Volumes and units correct on raw and final bench sheets
☐ Sample IDs have been checked—Bottle numbers appended if required
☐ Names present for: Started by, Completed by, relinquished by, and witnessed by.
☐ Training has been circled yes or no
☐ Extract Storage recorded
☐ Additional Prep Sheet completely filled out (NA or line out Blanks)
☐ Signed service request with Form V, if applicable, has been attached

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Columbia Analytical Services, INC.
Appendix from EXT-3541
For extracting 1653's in Solids by EPA Method 3541

Procedure:

1. Run the rinse cycle on Soxtherms prior to sample extraction. See EXT-3541 SOP and Operation Manual for programming instructions

2. All lab equipment that will have contact with the sample must be thoroughly rinsed with the extraction solvent.

3. Weigh approximately ***10 grams*** dry weight into extraction thimble. Record weight using all figures displayed on the scale.

4. Use DCM rinsed granular anhydrous Na_2SO_4 [a] for the MB and OPRS.

5. Add surrogates and matrix spikes. See chart below. **(Witnessed by a trained analyst)**

6. Place sample on Soxtherm and run the associated program. See EXT-3541 SOP and Operation Manual for programming instructions.

7. Be careful that the extract does not go dry. The goal is a volume of 10 to 20 ml. after reductions. Samples are removed and immediately covered in foil as soon as this level is reached to allow other sample to continue the reduction step. Additional reduction may be required.

8. Quantitatively transfer the extract to culture tubes.

9. Concentrate extract to 1 or 2 mL, solvent exchanging to ACN.

10. Add extract to 1 liter of DI water.

11. Allow samples to homogenize by spinning on stir plate for 15 minutes.

Derivitization steps 12-14 must proceed rapidly, one sample at a time.

12. Increase the speed of the stirring bar until the vortex is drawn to the bottom of the beaker.

13. Add 25mL of K_2CO_3 solution to spinning sample.

14. **IMMEDIATELY** add 25mL of Acetic Anhydride and spin for three to five minutes to complete the derivitization.

15. Transfer samples and add 200mL of Hexane to a 2L seperatory funnel, and shake vigorously for 2 minutes with periodic venting.

16. Allow phases to separate for a minimum of 10 minutes. Drain water back into beaker and collect the hexane in a 1000mL KD apparatus through a small layer of Na_2SO_4. It may be necessary to break up any emulsions in a centrifuge prior to adding to the KD.

17. Repeat steps 15-16 using 100mL of Hexane, two additional times.

18. Concentrate the extracts on an S-Evap with the temperature set between 95-100° C. Remove the KD when the solvent level is low enough to remove the collector. Extreme caution must be used to ensure that the sample does not concentrate too low or to dryness.

19. Concentrate the extracts to 0.5mL final volume in Hexane. Place in a labeled, amber 2mL autosampler vial.

Test	Initial Weight	Surrogates added	Spikes Added	Vial Color	Calculated Final Volume
1653 Solid/ Paperboards	10 grams dry	1653 Labeled Std: Acetone/1 mL + Methanol/1 mL	1653 Secondary Std: Acetone/500 µL + Methanol/500 µL	amber	0.5 mL

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Additional Prep Information For EPA 3541

OP Pesticides

Service Request: _____ **Workgroup:** _____

Sulfate Lot #:_____ DCM (GC^2)Lot #:_____

Date/Time/Initials Weighed:_____ Balance ID:_____ Calibration Verified ☐

Storage Location (if not extracted same day):_____

Soxtherm Start (Time/Date/Initial): _____

Soxtherm Stop (Time/Date/Initial): _____

N-Evap (Temp/ID/Time/Date/Initial): _____

GPC Clean-up (3640): Start _____ (Time/Date/Initial)

Stop _____ (Time/Date/Initial)

S-Evap (Temp/ID/Time/Date/Initial): _____

Solvent Exchange to Hexane (Time/Date/Initial/Lot#):_____

N-Evap (Temp/ID/Time/Date/Initial): _____

Acetone Lot#:_____

Extract Storage: _____

Completed (Time/Date/Initial): _____

Comments/Observations:

_____

_____

_____

_____

Bench Sheet Review Check List
❑ Hold Times Met (if no, Reason: _____)
❑ Prep date, dept, method, product code correct in stealth
❑ Spike Information correct
❑ Weights/Volumes and units correct on raw and final bench sheets
❑ Sample IDs have been checked—Bottle numbers appended if required
❑ Names present for: Started by, Completed by, relinquished by, and witnessed by.
❑ Training has been circled
❑ Extract Storage recorded
❑ Additional Prep Sheet completely filled out (NA or line out Blanks)
❑ All clean-ups have been noted on additional prep sheet
❑ Signed service request with Form V, if applicable, has been attached

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COLUMBIA ANALYTICAL SERVICES, INC.
Appendix from EXT-3541
for Extracting OP Pesticides in Soil or Tissue by
EPA Method 3541

Procedure:

1.Run the rinse cycle on Soxtherms prior to sample extraction. See EXT-3541 SOP and Operation Manual for programming instructions. ***Note:*** *Tissue glassware must be baked prior to use.*
Prog.=01, Temp. limit=200°, Ext. temp.=140°, Boil time=10m., Red. A=1x15ml., Ext. time=10m., Red B=1x15ml., sol. cool=30m., Red. int.=5m., Red pulse=5s., time 56m.

2. All lab equipment that will have contact with the sample must be thoroughly rinsed with the extraction solvent.

3. Thoroughly mix sample. Remove large rocks, sticks, leaves and other organic matter unless this is the primary make-up of the sample. Weigh approximately *10 grams* dry weight into a 150ml beaker. Record weight using all figures displayed on the scale.

4. Use granular anhydrous Na_2SO_4 [a] for the MB, LCS, and DLCS.

5. Mix sample with DCM rinsed anhydrous Na_2SO_4 [a] until sample is sandy and free flowing. Be careful with amount of sulfate used so as not to overload thimble capacity. Up to two thimbles may be used. If two thimbles are needed, split extracts are then combined during reduction.

6. Transfer dried extract to assembled Soxtherm thimble. Be careful not to contaminate rinsed labware.

7. Add *100µL* OP surrogate and *100µL* OP matrix spikes. **(Witnessed by a trained analyst)**

8. Add enough DCM to cover samples completely.

9. Place sample on Soxtherm and run the associated program. See EXT-3541 SOP and Operation Manual for programming instructions.
Prog.=04, Temp. limit=200°, Ext. temp.=140°, Boil time=1h., Red. A=3x15ml., Ext. time=1h., Red B=6x15ml., sol. cool=30m., Red. int.=5m., Red pulse=5s., time 3h3m.

10. Be careful that the extract does not go dry. The goal is a volume of 10 to 20 ml. after reductions. Samples are removed and immediately covered in foil as soon as this level is reached to allow other sample to continue the reduction step. Additional reduction may be required.

11. Quantitatively transfer the extract to culture tubes.

12. Take Sample to an intermediate volume of 10 mL.

13. Filter all 10 mL of sample using a 0.45µm filter.

14. Load sample on GPC using BNA program making sure all reservoirs are full and prior clean cycle has been completed.

15. Concentrate extracts on S-Evap. Temperature should be between 70-75° C.

16. Solvent Exchange to hexane on S-Evap.

17. Concentrate the extracts to *≤ 10 mL calculated final volume* on the N-Evap under a **gentle** stream of nitrogen with the temperature not exceeding 35°.

18. Take extracts to 5 mL for a calculated true final volume of 10ml in 1:1 Hexane/Acetone and place in labeled, amber 2mL autosampler vial. Extreme caution must be used to ensure that the sample does not concentrate too low or to dryness.
Note: When GPC cleanup is performed, only half of the original extract is retained. Therefore the extract must be concentrated to ½ the final volume to achieve the correct "calculated" final volume.

19. Complete all necessary paperwork.

If this SOP is accessed electronically, it is an uncontrolled copy and will not be updated.

Additional Prep Information For EPA 3541

PAH ALK

Service Request _____ **Workgroup** _____

Sulfate Lot#:_____ DCM (GC^2)Lot#:_____

Date/Time/Initials Weighed:_____ Balance ID:_____ Calibration Verified ☐

Storage Location (if not extracted same day):_____

Soxtherm Start (Time/Date/Initial): _____

Soxtherm Stop (Time/Date/Initial): _____

N-Evap (Temp/ID/Time/Date/Initial): _____

Hexane Exchange for Silica Gel(Time/Date/Initial): _____

Hexane Lot#:_____

Silica Gel Clean-up (3630)(Time/Date/Initial): _____

Silica Column Lot#:_____

1:1 Pentane/DCM Reagent Lot#:_____

Turbo Vap (Temp/ID/Time/Date/Initial): _____

Extract Storage: _____

Completed (Time/Date/Initial): _____

Comments/Observations:

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Bench Sheet Review Check List
❑ Hold Times Met (if no, Reason: _____)
❑ Prep date, dept, method, product code correct in stealth
❑ Spike Information correct
❑ Weights/Volumes and units correct on raw and final bench sheets
❑ Sample IDs have been checked—Bottle numbers appended if required
❑ Names present for: Started by, Completed by, relinquished by, and witnessed by.
❑ Training has been circled
❑ Extract Storage recorded
❑ Additional Prep Sheet completely filled out (NA or line out Blanks)
❑ All clean-ups have been noted on additional prep sheet
❑ Signed service request with Form V, if applicable, has been attached

If this SOP is accessed electronically, it is an uncontrolled copy and will not be updated.

COLUMBIA ANALYTICAL SERVICES, INC.
Appendix from EXT-3541
for Co-extracting PAHs and DROs and PAH Alks in Soil or Tissue by
EPA Method 3541

Procedure:

1. For low level work follow the low level glassware cleaning procedure.
2. Run the rinse cycle on Soxtherms prior to sample extraction. See EXT-3541 SOP and Operation Manual for programming instructions. ***Note:*** *For UL and Tissues, baked glassware must be used.*
Prog.=01, Temp. limit=200°, Ext. temp.=140°, Boil time=10m., Red. A=1x15ml., Ext. time=10m., Red B=1x15ml., sol. cool=30m., Red. int.=5m., Red pulse=5s., time 56m.
3. All lab equipment that will have contact with the sample must be thoroughly rinsed with the extraction solvent.
4. Thoroughly mix sample. Remove large rocks, sticks, leaves and other organic matter unless this is the primary make-up of the sample. Weigh approximately (see chart) *initial weight* into a 150ml beaker. Record weight using all figures displayed on the scale.
5. Use granular anhydrous Na_2SO_4 [a] for the MB, LCS, and DLCS. For sediments and tissues weigh 5 grams of the appropriate SRM for analysis if requested on the service request.
6. Mix sample with DCM rinsed anhydrous Na_2SO_4 [a] until sample is sandy and free flowing. Be careful with amount of sulfate used so as not to overload thimble capacity. Up to two thimbles may be used. If two thimbles are needed, split extracts are then combined during reduction.
7. Transfer dried extract to assembled Soxtherm thimble. Be careful not to contaminate rinsed labware.
8. Add surrogate and matrix spikes. See chart. **(Witnessed by a trained analyst)**
9. Add enough DCM to cover samples completely.
10. Place sample on Soxtherm and run the associated program. See EXT-3541 SOP and Operation Manual for programming instructions.
Prog.=04, Temp. limit=200°, Ext. temp.=140°, Boil time=1h., Red. A=3x15ml., Ext. time=1h., Red B=6x15ml., sol. cool=30m., Red. int.=5m., Red pulse=5s., time 3h3m.
11. Be careful that the extract does not go dry. The goal is a volume of 10 to 20 ml. after reductions. Samples are removed and immediately covered in foil as soon as this level is reached to allow other sample to continue the reduction step. Additional reduction may be required.
12. Quantitatively transfer the extract to culture tubes.
13. Take Sample to an *intermediate volume* (see chart). Split extracts into two equal aliquots with one aliquot relinquished to Semivoa GC for DRO clean-ups if co-extracted.
Note: *No clean-ups are necessary for puffs and filters. GCMS aliquot is taken to 2.5mL for a calculated final volume of 5 mL.*
14. Place 1mL of extract from GCMS aliquot into a blue vial for DPnB/Bio analysis if needed. Pull off 1mL of extract from GCMS aliquot and solvent exchange into hexane by adding ~1mL of hexane to extract and concentrating back down to ~1mL on N-evap. Repeat two more times.
15. Condition silica gel columns 3 times with DCM and 3 times with hexane.
16. Place a test tube under column and add extract to silica gel column.
17. Elute 5 times with 1:1 DCM/ Pentane.
18. Concentrate the extracts to ~1mL on turbo vap and bring to 1mL.
19. Place 1mL of extract into PAH vials.
20. Complete all necessary paperwork.

Test	Initial Weight	Surrogate Added	Spike Added	Intermediate Volume	Vial Color	Calculated Final Volume
PAH ALK Soils	10 grams dry	AP / 20µL	PAH / 200µL DPnB / 400 µL	10 mL	PAH / Green BIO / Blue	10 mL
PAH ALK tissues	10 grams	AP / 20µL	PAH / 100µL Corn oil/100 µL* DPnB 200uL	5 mL	PAH / Pink BIO / Blue	5 mL
SIM-PAH / PAH-ALK **co-extract**	20 grams dry	AP / 40µL SVF / 200 µL	PAH / 400 µL DPnB/800uL	20 mL	PAH / Green BIO / Blue	20 mL
SIM-PAH Puffs and Filters **co-extract**	1 puff or filter	AP / 20 µL SVF / 100 µL	PAH / 80 µL DPnB 200uL	10 mL	PAH/Green BIO/Blue	5 mL
SIM-PAH Filters and VOAs** **Co-extract**	1 filter	AP / 10 µL SVF / 50 µL	PAH / 40 µL DPnB 100uL	10 mL	PAH/Pink BIO/Blue	5 mL

*Add corn oil to MB/LCS/DLCS
**Filter extracted by 3541 then combined with VOA portion, each portion receiving the surrogates and spikes.

If this SOP is accessed electronically, it is an uncontrolled copy and will not be updated.

Additional Prep Information For EPA 3541

OC Pesticides by GCMS

Service Request _____ **Workgroup** _____

Sulfate Lot #_____ DCM (GC^2)Lot #_____

Date/Time/Initials Weighed:_____ Balance ID:_____ Calibration Verified ☐

Storage Location (if not extracted same day):_____

Soxtherm Start (Time/Date/Initial): _____

Soxtherm Stop (Time/Date/Initial): _____

N-Evap (Temp/ID/Time/Date/Initial): _____

GPC Clean-up (3640): Start _____ (Time/Date/Initial)

Stop _____ (Time/Date/Initial)

S-Evap (Temp/ID/Time/Date/Initial): _____

N-Evap (Temp/ID/Time/Date/Initial): _____

Hexane Exchange for Carbon(Time/Date/Initial): _____

Hexane Lot #_____

Carbon Clean-up (3630)(Time/Date/Initial): _____

Carbon Column Lot #_____ 4:1 Hexane / DCM Lot # _____

N-Evap (Temp/ID/Time/Date/Initial): _____

Extract Storage: _____

Completed (Time/Date/Initial): _____

Comments/Observations:

_____

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_____

Bench Sheet Review Check List
❑ Hold Times Met (if no, Reason: _____)
❑ Prep date, dept, method, product code correct in stealth
❑ Spike Information correct
❑ Weights/Volumes and units correct on raw and final bench sheets
❑ Sample IDs have been checked—Bottle numbers appended if required
❑ Names present for: Started by, Completed by, relinquished by, and witnessed by.
❑ Training has been circled
❑ Extract Storage recorded
❑ Additional Prep Sheet completely filled out (NA or line out Blanks)
❑ All clean-ups have been noted on additional prep sheet
❑ Signed service request with Form V, if applicable, has been attached

If this SOP is accessed electronically, it is an uncontrolled copy and will not be updated.

COLUMBIA ANALYTICAL SERVICES, INC.
Appendix from EXT-3541
for Extracting OC Pesticides by GCMS in Soil or Tissue by
EPA Method 3541

Procedure:

1.Run the rinse cycle on Soxtherms prior to sample extraction. See EXT-3541 SOP and Operation Manual for programming instructions. ***Note:*** *For UL and Tissues, baked glassware must be used.*
Prog.=01, Temp. limit=200°, Ext. temp.=140°, Boil time=10m., Red. A=1x15ml., Ext. time=10m., Red B=1x15ml., sol. cool=30m., Red. int.=5m., Red pulse=5s., time 56m.

2. All lab equipment that will have contact with the sample must be thoroughly rinsed with the extraction solvent.

3. Thoroughly mix sample. Remove large rocks, sticks, leaves and other organic matter unless this is the primary make-up of the sample. Weigh approximately ***10 grams*** dry weight for soils, ***2grams*** dry weight for tissues, into a 150ml beaker. Record weight using all figures displayed on the scale.

4. Use granular anhydrous Na_2SO_4 [a] for the MB, LCS, and DLCS.

5. Mix sample with DCM rinsed anhydrous Na_2SO_4 [a] until sample is sandy and free flowing. Be careful with amount of sulfate used so as not to overload thimble capacity. Up to two thimbles may be used. If two thimbles are needed, split extracts are then combined during reduction.

6. Transfer dried extract to assembled Soxtherm thimble. Be careful not to contaminate rinsed labware.

7. Add ***100 µL*** OCPest surrogate and ***100 µL*** OCPest matrix spike. **(Witnessed by a trained analyst)**

8. Add enough DCM to cover samples completely.

9. Place sample on Soxtherm and run the associated program. See EXT-3541 SOP and Operation Manual for programming instructions.
Prog.=04, Temp. limit=200°, Ext. temp.=140°, Boil time=1h., Red. A=3x15ml., Ext. time=1h., Red B=6x15ml., sol. cool=30m., Red. int.=5m., Red pulse=5s., time 3h3m.

10. Be careful that the extract does not go dry. The goal is a volume of 10 to 20 ml. after reductions. Samples are removed and immediately covered in foil as soon as this level is reached to allow other sample to continue the reduction step. Additional reduction may be required.

11. Quantitatively transfer the extract to culture tubes.

12. **For tissues:** Concentrate extracts to 10 mL and perform GPC clean-up

13. Concentrate extract to ~ 1 mL.

14. Solvent exchange into hexane by adding ~1mL of hexane to extract and concentrating back down to ~1mL on N-Evap. Repeat two more times.

15. Perform a carbon clean-up on the extract.

16. Concentrate extracts on N-Evap and bring back to ***1 mL final volume*** in hexane. Place in a 2mL amber autosampler vial.

17. Complete all necessary paperwork.

If this SOP is accessed electronically, it is an uncontrolled copy and will not be updated.

Additional Prep Information For EPA 3541

Nonylphenols

Service Request _____ **Workgroup** _____

Sulfate Lot #:_____ DCM (GC^2) Lot #:_____

Date/Time/Initials Weighed:_____ Balance ID:_____ Calibration Verified ☐

Storage Location (if not extracted same day):_____

Soxtherm Start (Time/Date/Initial): _____

Soxtherm Stop (Time/Date/Initial): _____

N-Evap (Temp/ID/Time/Date/Initial): _____

Solvent Exchange to ACN (Time/Date/Initial): _____

ACN Lot #_____

Turbo Vap (Temp/ID/Time/Date/Initial): _____

Mg Sulfate lot #_____

Extract Storage: _____

Completed(Time/Date/Initial): _____

Comments/Observations:

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Bench Sheet Review Check List
☐ Hold Times Met (if no, Reason: _____)
☐ Prep date, dept, method, product code correct in stealth
☐ Spike Information correct
☐ Weights/Volumes and units correct on raw and final bench sheets
☐ Sample IDs have been checked—Bottle numbers appended if required
☐ Names present for: Started by, Completed by, relinquished by, and witnessed by.
☐ Training has been circled
☐ Extract Storage recorded
☐ Additional Prep Sheet completely filled out (NA or line out Blanks)
☐ All clean-ups have been noted on additional prep sheet
☐ Signed service request with Form V, if applicable, has been attached

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COLUMBIA ANALYTICAL SERVICES, INC.
Appendix from EXT-3541
for Extracting Nonylphenols in Solids by
EPA Method 3541

Procedure:

1.Run the rinse cycle on Soxtherms prior to sample extraction. See EXT-3541 SOP and Operation Manual for programming instructions.
Prog.=01, Temp. limit=200°, Ext. temp.=140°, Boil time=10m., Red. A=1x15ml., Ext. time=10m., Red B=1x15ml., sol. cool=30m., Red. int.=5m., Red pulse=5s., time 56m.

2. All lab equipment that will have contact with the sample must be thoroughly rinsed with the extraction solvent.

3. Thoroughly mix sample. Remove large rocks, sticks, leaves and other organic matter unless this is the primary make-up of the sample. Weigh approximately 10 grams dry weight(soils) or 1gram for paperboards into a 150ml beaker. Record weight using all figures displayed on the scale.

4. Use granular anhydrous Na_2SO_4 [a] for the MB, LCS and DLCS.

5. Mix sample with DCM rinsed anhydrous Na_2SO_4 [a] until sample is sandy and free flowing. Be careful with amount of sulfate used so as not to overload thimble capacity. Up to two thimbles may be used. If two thimbles are needed, split extracts are then combined during reduction.

6. Transfer dried extract to assembled Soxtherm thimble. Be careful not to contaminate rinsed labware.

7. Add **100 µL** of Nonylphenol surrogate and **100 µL** of Nonylphenol matrix spike to samples. **(Witnessed by a trained analyst)**

8. Add enough DCM to cover samples completely.

9. Place sample on Soxtherm and run the associated program. See EXT-3541 SOP and Operation Manual for programming instructions.
Prog.=04, Temp. limit=200°, Ext. temp.=140°, Boil time=1h., Red. A=3x15ml., Ext. time=1h., Red B=6x15ml., sol. cool=30m., Red. int.=5m., Red pulse=5s., time 3h3m.

10. Be careful that the extract does not go dry. The goal is a volume of 10 to 20 ml. after reductions. Samples are removed and immediately covered in foil as soon as this level is reached to allow other sample to continue the reduction step. Additional reduction may be required.

11. Quantitatively transfer the extract to culture tubes.

12. Take Sample to a final volume of 5 mLs.

13. Pull off 1 mL (for samples that are extremely "dirty" use a 200uL aliquot and proceed) of extract and add 4 mLs of ACN. Let extracts sit for at least 1 hour and filter.

14. Concentrate extracts on Turbo Vap and bring to 1 mL.

15. Add a pinch of magnesium sulfate and vortex extract for 5 seconds to dry the extract.

16. Use a 3 mL 0.45µm mesh filter to filter extract into a 2 mL amber autosampler vial.

17.Complete all necessary paperwork.

If this SOP is accessed electronically, it is an uncontrolled copy and will not be updated.

Additional Prep Information For EPA 3541

PAH

Service Request _____ **Workgroup** _____

Sulfate Lot #_____ DCM (GC^2)Lot #_____

Date/Time/Initials Weighed:_____ Balance ID:_____ Calibration Verified ☐

Storage Location (if not extracted same day):_____

Soxtherm Start (Time/Date/Initial): _____

Soxtherm Stop (Time/Date/Initial): _____

N-Evap (Temp/ID/Time/Date/Initial): _____

Hexane Exchange for Silica Gel(Time/Date/Initial): _____
Hexane Lot #_____

Silica Gel Clean-up (3630)(Time/Date/Initial): _____

Silica Column Lot #_____ 1:1 Hexane/DCM Reagent Lot #_____

Turbo Vap (Temp/ID/Time/Date/Initial): _____

Extract Storage: _____

Completed (Time/Date/Initial): _____

Comments/Observations:

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Bench Sheet Review Check List
☐ Hold Times Met (if no, Reason: _____)
☐ Prep date, dept, method, product code correct in stealth
☐ Spike Information correct
☐ Weights/Volumes and units correct on raw and final bench sheets
☐ Sample IDs have been checked—Bottle numbers appended if required
☐ Names present for: Started by, Completed by, relinquished by, and witnessed by.
☐ Training has been circled
☐ Extract Storage recorded
☐ Additional Prep Sheet completely filled out (NA or line out Blanks)
☐ All clean-ups have been noted on additional prep sheet
☐ Signed service request with Form V, if applicable, has been attached

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COLUMBIA ANALYTICAL SERVICES, INC.
Appendix from EXT-3541
for Extracting PAHs in Soil or Tissue by
EPA Method 3541

Procedure:

1. For low level work follow the low level glassware cleaning procedure.
2. Run the rinse cycle on Soxtherms prior to sample extraction. See EXT-3541 SOP and Operation Manual for programming instructions. ***Note:*** *For UL and Tissues, baked glassware must be used.*
Prog.=01, Temp. limit=200°, Ext. temp.=140°, Boil time=10m., Red. A=1x15ml., Ext. time=10m., Red B=1x15ml., sol. cool=30m., Red. int.=5m., Red pulse=5s., time 56m.
3. All lab equipment that will have contact with the sample must be thoroughly rinsed with the extraction solvent.
4. Thoroughly mix sample. Remove large rocks, sticks, leaves and other organic matter unless this is the primary make-up of the sample. Weigh approximately (see chart) *initial weight* into a 150ml beaker. Record weight using all figures displayed on the scale.
5. Use granular anhydrous Na_2SO_4 [a] for the MB, LCS, and DLCS. For sediments and tissues weigh 5 grams of the appropriate SRM for analysis if requested on the service request.
6. Mix sample with DCM rinsed anhydrous Na_2SO_4 [a] until sample is sandy and free flowing. Be careful with amount of sulfate used so as not to overload thimble capacity. Up to two thimbles may be used. If two thimbles are needed, split extracts are then combined during reduction.
7. Transfer dried extract to assembled Soxtherm thimble. Be careful not to contaminate rinsed labware.
8. Add surrogate and matrix spikes. See chart. **(Witnessed by a trained analyst)**
9. Add enough DCM to cover samples completely.
10. Place sample on Soxtherm and run the associated program. See EXT-3541 SOP and Operation Manual for programming instructions.
Prog.=04, Temp. limit=200°, Ext. temp.=140°, Boil time=1h., Red. A=3x15ml., Ext. time=1h., Red B=6x15ml., sol. cool=30m., Red. int.=5m., Red pulse=5s., time 3h3m.
11. Be careful that the extract does not go dry. The goal is a volume of 10 to 20 ml. after reductions. Samples are removed and immediately covered in foil as soon as this level is reached to allow other sample to continue the reduction step. Additional reduction may be required.
12. Quantitatively transfer the extract to culture tubes.
13. Take Sample to an intermediate volume.
14. Pull off 1mL of extract and solvent exchange into hexane by adding ~1mL of hexane to extract and concentrating back down to ~1mL on N-evap. Repeat two more times.
15. Condition silica gel columns 3 times with DCM and 3 times with hexane.
16. Place a test tube under column and add extract to silica gel column.
17. Elute 4 times with 1:1 DCM/ Hexane.
18. Concentrate the extracts to 1mL on turbo vap. Place 1 mL of extract into PAH vials.
19. Complete all necessary paperwork.

Test	Initial Weight	Surrogate Added	Spike Added	Vial Color	Calculated Final Volume
SIM-PAH/ SIMALK Soils/Paperboards	10 grams dry	AP / 20 µL	PAH / 200 µL	Green	10 mL
SIM-PAH UL Solids	20 grams dry	AP / 20 µL	PAH / 80 µL	Green	2 mL
SIM-PAH UL Tissue	10 grams	AP / 20 µL	PAH / 100 µL*	Pink	1 mL
SIM-PAH Tissue	10 grams	AP / 20 µL	PAH / 100 µL*	Pink	5 mL
SIM-PAH Wipe	1 wipe	AP / 10 µL	PAH / 20 µL	Green	1 mL

* *Add 100 µL of corn oil to the LCS, DLCS, and MB*

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Additional Prep Information For EPA 3541

SIM-PAH-PCP

Service Request _____ **Workgroup** _____

Sulfate Lot #_____ DCM (GC^2)Lot #_____

Date/Time/Initials Weighed:_____ Balance ID:_____ Calibration Verified ☐

Storage Location (if not extracted same day):_____

Soxtherm Start (Time/Date/Initial): _____

Soxtherm Stop (Time/Date/Initial): _____

N-Evap (Temp/ID/Time/Date/Initial): _____

GPC Clean-up (3640): Start _____ (Time/Date/Initial)
Stop _____ (Time/Date/Initial)

S-Evap (Temp/ID/Time/Date/Initial): _____

N-Evap (Temp/ID/Time/Date/Initial): _____

Extract Storage: _____

Completed (Time/Date/Initial): _____

Comments/Observations:

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Bench Sheet Review Check List
❑ Hold Times Met (if no, Reason: _____)
❑ Prep date, dept, method, product code correct in stealth
❑ Spike Information correct
❑ Weights/Volumes and units correct on raw and final bench sheets
❑ Sample IDs have been checked—Bottle numbers appended if required
❑ Names present for: Started by, Completed by, relinquished by, and witnessed by.
❑ Training has been circled
❑ Extract Storage recorded
❑ Additional Prep Sheet completely filled out (NA or line out Blanks)
❑ All clean-ups have been noted on additional prep sheet
❑ Signed service request with Form V, if applicable, has been attached

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COLUMBIA ANALYTICAL SERVICES, INC.
Appendix from EXT-3541
for Extracting SIM-PAH-PCPs in Soil or Tissue by
EPA Method 3541

Procedure:

1..Run the rinse cycle on Soxtherms prior to sample extraction. See EXT-3541 SOP and Operation Manual for programming instructions.
Prog.=01, Temp. limit=200°, Ext. temp.=140°, Boil time=10m., Red. A=1x15ml., Ext. time=10m., Red B=1x15ml., sol. cool=30m., Red. int.=5m., Red pulse=5s., time 56m.

2. All lab equipment that will have contact with the sample must be thoroughly rinsed with the extraction solvent.

3. Thoroughly mix sample. Remove large rocks, sticks, leaves and other organic matter unless this is the primary make-up of the sample. Weigh approximately *10 grams dry* weight into a 150ml beaker. Record weight using all figures displayed on the scale.

4. Use granular anhydrous Na_2SO_4 [a] for the MB, LCS, and DLCS. For sediments and tissues weigh 5 grams of the appropriate SRM for analysis if requested on the service request.

5. Mix sample with DCM rinsed anhydrous Na_2SO_4 [a] until sample is sandy and free flowing. Be careful with amount of sulfate used so as not to overload thimble capacity. Up to two thimbles may be used. If two thimbles are needed, split extracts are then combined during reduction.

6. Transfer dried extract to assembled Soxtherm thimble. Be careful not to contaminate rinsed labware.

7. Add surrogate and matrix spikes. See chart. **(Witnessed by a trained analyst)**

8. Add enough DCM to cover samples completely.

9. Place sample on Soxtherm and run the associated program. See EXT-3541 SOP and Operation Manual for programming instructions.
Prog.=04, Temp. limit=200°, Ext. temp.=140°, Boil time=1h., Red. A=3x15ml., Ext. time=1h., Red B=6x15ml., sol. cool=30m., Red. int.=5m., Red pulse=5s., time 3h3m.

10. Be careful that the extract does not go dry. The goal is a volume of 10 to 20 ml. after reductions. Samples are removed and immediately covered in foil as soon as this level is reached to allow other sample to continue the reduction step. Additional reduction may be required.

11. Quantitatively transfer the extract to culture tubes.

12. Take Sample to an intermediate volume of 10 mLs.

13. Filter all 10 mLs of sample using a 0.45µm filter.

14. Load sample on GPC using BNA program making sure all reservoirs are full and prior clean cycle has been completed.

15. Concentrate extracts on S-Evap. Temperature should be between 70-75° C.

16. Concentrate the extracts to 5mL on the N-Evap under a **gentle** stream of nitrogen with the temperature not exceeding 35° and place in labeled, green 2mL autosampler vial. Extreme caution must be used to ensure that the sample does not concentrate too low or to dryness.
Note: When GPC cleanup is performed, only half of the original extract is retained. Therefore the extract must be concentrated to ½ the final volume to achieve the correct "calculated" final volume.

17.Complete all necessary paperwork.

Test	Initial Weight	Surrogate Amount	Spike Amount	Vial Color	Calculated Final Volume
SIM-PAH-PCP Soil or Tissue	10 grams dry	AP / 20µL	PAH / 200 µL PCP / 400 µL	Green	10 mL

If this SOP is accessed electronically, it is an uncontrolled copy and will not be updated.

Additional Prep Information For EPA 3541

PBDE

Service Request _____ **Workgroup** _____

Sulfate Lot #_____ DCM (GC^2)Lot #_____

Date/Time/Initials Weighed:_____ Balance ID:_____ Calibration Verified ☐

Storage Location (if not extracted same day):_____

Soxtherm Start (Time/Date/Initial): _____

Soxtherm Stop (Time/Date/Initial): _____

N-Evap (Temp/ID/Time/Date/Initial): _____

Hexane Exchange for Silica Gel (Time/Date/Initial): _____
Hexane Lot #_____

Silica Gel Clean-up (3630)(Time/Date/Initial): _____

Silica Column Lot #_____ 1:1 Hexane/DCM Reagent Lot #_____

N-Evap (Temp/ID/Time/Date/Initial): _____

Sulfuric Acid Clean-up (Time/Date/Initial): _____ Acid Lot #_____

Extract Storage: _____

Completed(Time/Date/Initial): _____

Comments/Observations:

_____

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_____

_____

Bench Sheet Review Check List
❑ Hold Times Met (if no, Reason: _____)
❑ Prep date, dept, method, product code correct in stealth
❑ Spike Information correct
❑ Weights/Volumes and units correct on raw and final bench sheets
❑ Sample IDs have been checked—Bottle numbers appended if required
❑ Names present for: Started by, Completed by, relinquished by, and witnessed by.
❑ Training has been circled
❑ Extract Storage recorded
❑ Additional Prep Sheet completely filled out (NA or line out Blanks)
❑ All clean-ups have been noted on additional prep sheet
❑ Signed service request with Form V, if applicable, has been attached

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COLUMBIA ANALYTICAL SERVICES, INC.
Appendix from EXT-3541
for Extracting PBDEs in Soil or Tissue by
EPA Method 3541

Procedure:

1. For low level work follow the low level glassware cleaning procedure.
2. Run the rinse cycle on Soxtherms prior to sample extraction. See EXT-3541 SOP and Operation Manual for programming instructions. *Note: For UL and Tissues, baked glassware must be used.*
Prog.=01, Temp. limit=200°, Ext. temp.=140°, Boil time=10m., Red. A=1x15ml., Ext. time=10m., Red B=1x15ml., sol. cool=30m., Red. int.=5m., Red pulse=5s., time 56m.
3. All lab equipment that will have contact with the sample must be thoroughly rinsed with the extraction solvent.
4. Thoroughly mix sample. Remove large rocks, sticks, leaves and other organic matter unless this is the primary make-up of the sample. Weigh approximately (see chart) *initial weight* into a 150ml beaker. Record weight using all figures displayed on the scale.
5. Use granular anhydrous Na_2SO_4 [a] for the MB, LCS, and DLCS. For sediments and tissues weigh 5 grams of the appropriate SRM for analysis if requested on the service request.
6. Mix sample with DCM rinsed anhydrous Na_2SO_4 [a] until sample is sandy and free flowing. Be careful with amount of sulfate used so as not to overload thimble capacity. Up to two thimbles may be used. If two thimbles are needed, split extracts are then combined during reduction.
7. Transfer dried extract to assembled Soxtherm thimble. Be careful not to contaminate rinsed labware.
8. Add surrogate and matrix spikes. See chart. **(Witnessed by a trained analyst)**
9. Add enough DCM to cover samples completely.
10. Place sample on Soxtherm and run the associated program. See EXT-3541 SOP and Operation Manual for programming instructions.
Prog.=04, Temp. limit=200°, Ext. temp.=140°, Boil time=1h., Red. A=3x15ml., Ext. time=1h., Red B=6x15ml., sol. cool=30m., Red. int.=5m., Red pulse=5s., time 3h3m.
11. Be careful that the extract does not go dry. The goal is a volume of 10 to 20 ml. after reductions. Samples are removed and immediately covered in foil as soon as this level is reached to allow other sample to continue the reduction step. Additional reduction may be required.
12. Quantitatively transfer the extract to culture tubes.
13. Take Sample to the *calculated final volume*.
14. Pull off 2 mL of extract and solvent exchange into hexane by adding ~ 2 mL of hexane to extract and concentrating back down to ~2 mL on N-evap. Repeat two more times.
15. Condition silica gel cartridges 2 times with DCM and 2 times with hexane.
16. Place test tubes in manifold and load samples.
17. Elute 2 times with 1:1 DCM/ Hexane.
18. Concentrate the extracts to 2mL on N-Evap.
19. Perform sulfuric acid clean-up.
20. Complete all necessary paperwork.

Test	Initial Weight	Surrogate Added	Spike Added	Vial Color	Calculated Final Volume
PBDE soil	10 grams dry	PBDE / 50µL	PBDE / 50 µL	Amber	2 mL
PBDE tissue	10 grams	PBDE / 50 µL	PBDE / 50 µL	Amber	2 mL
PBDE Paperboard	1 gram	PBDE / 100 µL	PBDE / 100 µL	Amber	10 mL

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Additional Prep Information For EPA 3541

8270 and 8270-LL

Service Request _____ **Workgroup** _____

Sulfate Lot #_____ DCM (GC^2)Lot #_____

Date/Time/Initials Weighed:_____ Balance ID:_____ Calibration Verified ☐

Storage Location (if not extracted same day):_____

Soxtherm Start (Time/Date/Initial): _____

Soxtherm Stop (Time/Date/Initial): _____

N-Evap (Temp/ID/Time/Date/Initial): _____

GPC Clean-up (3640): Start _____ (Time/Date/Initial)

Stop _____ (Time/Date/Initial)

S-Evap (Temp/ID/Time/Date/Initial): _____

N-Evap (Temp/ID/Time/Date/Initial): _____

Extract Storage: _____

Completed (Time/Date/Initial): _____

Comments/Observations:

_____

_____

_____

_____

Bench Sheet Review Check List
❑ Hold Times Met (if no, Reason: _____)
❑ Prep date, dept, method, product code correct in stealth
❑ Spike Information correct
❑ Weights/Volumes and units correct on raw and final bench sheets
❑ Sample IDs have been checked—Bottle numbers appended if required
❑ Names present for: Started by, Completed by, relinquished by, and witnessed by.
❑ Training has been circled
❑ Extract Storage recorded
❑ Additional Prep Sheet completely filled out (NA or line out Blanks)
❑ All clean-ups have been noted on additional prep sheet
❑ Signed service request with Form V, if applicable, has been attached

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Appendix from EXT-3541
for Extracting 8270's and 8270-LL's in Soil or Tissue by
EPA Method 3541

Procedure:

1.Run the rinse cycle on Soxtherms prior to sample extraction. See EXT-3541 SOP and Operation Manual for programming instructions. *Note: Tissue glassware must be baked prior to use.*
Prog.=01, Temp. limit=200°, Ext. temp.=140°, Boil time=10m., Red. A=1x15ml., Ext. time=10m., Red B=1x15ml., sol. cool=30m., Red. int.=5m., Red pulse=5s., time 56m.

2. All lab equipment that will have contact with the sample must be thoroughly rinsed with the extraction solvent.

3. Thoroughly mix sample. Remove large rocks, sticks, leaves and other organic matter unless this is the primary make-up of the sample. Weigh approximately (see chart) *initial weight* into a 150ml beaker. Record weight using all figures displayed on the scale.

4. Use granular anhydrous Na_2SO_4 [a] for the MB, LCS, and DLCS. For sediments and tissues weigh 5 grams of the appropriate SRM for analysis if requested on the service request.

5. Mix sample with DCM rinsed anhydrous Na_2SO_4 [a] until sample is sandy and free flowing. Be careful with amount of sulfate used so as not to overload thimble capacity. Up to two thimbles may be used. If two thimbles are needed, split extracts are then combined during reduction.

6. Transfer dried extract to assembled Soxtherm thimble. Be careful not to contaminate rinsed labware.

7. Add surrogate and matrix spikes. See chart. **(Witnessed by a trained analyst)**

8. Add enough DCM to cover samples completely.

9. Place sample on Soxtherm and run the associated program. See EXT-3541 SOP and Operation Manual for programming instructions.
Prog.=04, Temp. limit=200°, Ext. temp.=140°, Boil time=1h., Red. A=3x15ml., Ext. time=1h., Red B=6x15ml., sol. cool=30m., Red. int.=5m., Red pulse=5s., time 3h3m.

10. Be careful that the extract does not go dry. The goal is a volume of 10 to 20 ml. after reductions. Samples are removed and immediately covered in foil as soon as this level is reached to allow other sample to continue the reduction step. Additional reduction may be required.

11. Quantitatively transfer the extract to culture tubes.

12. Take samples to an intermediate volume of 10 mLs.

13. Filter all 10 mLs of sample using a 0.45µm filter.

14. Load samples on GPC using BNA program making sure all reservoirs are full and prior clean cycle has been completed.

15. Concentrate extracts on S-Evap. Temperature should be between 70-75° C.

16. Concentrate the extracts to < *calculated final volume (see below)* on the N-Evap under a **gentle** stream of nitrogen with the temperature not exceeding 35° and place in labeled, colored 2mL autosampler vial. Extreme caution must be used to ensure that the sample does not concentrate too low or to dryness.
Note: When GPC cleanup is performed, only half of the original extract is retained. Therefore the extract must be concentrated to ½ the final volume to achieve the correct "calculated" final volume.

17.Complete all necessary paperwork.

Test	Initial Weight	Surrogate Amount	Spike Amount	Vial Color	Calculated Final Volume
8270-LL	20 grams dry	AP/50µL	8270/50 µL Benzoic Acid/ 50 µL	Amber	2 mL
8270	30 grams dry	AP/ 1mL	8270/ 1mL	Clear	1 mL
8270 Paperboards	20 grams	AP/ 1mL	8270/ 1mL Benzidine/ 100 µ L Paperboard/ 20 µL	Clear	1 mL
8270 Wipe	1 wipe	AP/ 1mL	8270/ 1mL	Clear	1 mL
SVO-SIM Tissue	5 grams	AP/50µL	8270/50 µL	Amber	10 mL

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STANDARD OPERATING PROCEDURE

PERCENT LIPIDS IN TISSUE

EXT-LIPID
Revision 2
September 20, 2010

Approved By:

Supervisor	8/30/10 Date
QA Manager	8/30/10 Date
Laboratory Manager	8/30/10 Date

COLUMBIA ANALYTICAL SERVICES, INC.
1317 South 13th Avenue
Kelso, Washington 98626

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Annual review of this SOP has been performed and the SOP still reflects current practice.
Initials: _____ Date: _____
Initials: _____ Date: _____
Initials: _____ Date: _____

DOCUMENT CONTROL
NUMBER: _____
Initials: _____ Date: _____

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PERCENT LIPIDS IN TISSUE

1. SCOPE AND APPLICATION

1.1. This procedure is used to determine the lipid content in biological tissue. The procedures used are based on the procedure described in the Puget Sound Protocols, Bligh & Dyer, and the EPA Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories.

1.2. This procedure is typically applied to marine tissues. In some cases, the procedure can be used to determine lipids in other biological tissue.

2. METHOD SUMMARY

2.1. A homogenized portion of the tissue sample is extracted with organic solvent. The extract is evaporated using moderate heat and the lipid weight is determined. Percent lipids is calculated from the weight measured.

2.2. The extraction may either be performed independently of the extraction done for other organics analyses or a portion of the extract from another extraction may be used for the percent lipids determination

3. DEFINITION

Lipids - Naturally occurring compounds that are soluble in such organic solvents as dichloromethane, hydrocarbons, chloroform, benzene, and alcohols. This includes a diverse range of compounds, like fatty acids and their derivatives, carotenoids, terpenes, steroids, cholesterols, and bile acids

4. INTERFERENCES

4.1. Since the procedure is used to provide an estimation of lipid content based on non-volatile extracted material, interferences from co-extracted components that are not considered lipids may occur.

4.2. Due to the nature of the procedure, interferences attributed to laboratory procedures are not typically encountered.

5. SAFETY

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Personal protective equipment will include safety glasses (with side shields), gloves, and a lab coat. Follow normal precautions as per the CAS Safety Manual.

6. SAMPLE HANDLING AND PRESERVATION

Tissue samples should stored at -20 ± 4°C until analysis. When frozen, samples should be analyzed within 1 year of sampling. Project-specific protocols may apply to sample storage and holding time requirements.

7. APPARATUS

7.1. Evaporating pans: Aluminum weighing pans.

7.2. VOA vials, 40mL, precleaned.

7.3. Tissuemizer.

7.4. Drying oven, capable of maintaining a temperature of 70°C.

7.5. Hotplate, capable of maintaining a temperature of 70°C.

7.6. Analytical balance, capable of weighing to 0.1mg.

8. REAGENTS

8.1. Sodium sulfate (granular, anhydrous), Na_2SO_4. Purify by heating at 400ºC for 4 hours in a shallow tray or crucible, or by pre-cleaning the sodium sulfate with dichloromethane.

8.2. Extraction solvents:

8.2.1. Dichloromethane

8.2.2. Chloroform/Methanol (1:1)

9. PREVENTIVE MAINTENANCE

9.1. Balance calibration checks are performed daily for each day analyses are performed. The results of these checks are recorded in the designated logbook for balance calibration verification located in the extractions lab.

9.2. Periodic balance servicing is performed by an outside qualified vendor.

10. RESPONSIBILITIES

It is the responsibility of the analyst to perform the analysis according to this SOP and to complete all documentation required for data review. Analysis and interpretation of the results are performed by personnel in the laboratory who have demonstrated the ability to generate

acceptable results utilizing this SOP. This demonstration is in accordance with the training program of the laboratory. Final review and sign-off of the data is performed by the department supervisor/manager or designee.

11. PROCEDURE

Note: If samples are suspected to be high in lipids (i.e. whole fish), the % lipids must be done before samples are run on GPC to prevent overloading of GPC column. The maximum amount of lipid that can be loaded on the GPC column is 2 g. If lipid is over 2g see the extraction supervisor for assistance.

11.1. PSEP Option:

11.1.1. Homogenize the tissue sample using the CAS *SOP Tissue Sample Preparation (MET-TISP)*. If using a portion of extract from an analytical extraction, use 2 ml of extract from the 10 ml intermediate volume before GPC cleanup and skip to section 11.1.6.

11.1.2. Weigh 2 grams of tissue into a VOA vial. Dry the sample with sodium sulfate.

11.1.3. Add 10mL dichloromethane.

11.1.4. Using a tissuemizer, homogenize the tissue/solvent mixture until sample is thoroughly mixed.

11.1.5. Vortex and centrifuge at 100xG for 10 minutes.

11.1.6. Heat sample pans in oven at 70ºC for one hour. Remove from oven and weigh as soon as pans are no longer warm (less than 5 minutes). Weigh the pans to the nearest 0.0001 gram.

11.1.7. Pipet 2mL of the extract into a DCM-rinsed glass syringe with a new filter. Filter the extract into a pan and evaporate to dryness. Using a hot plate with temperature <70ºC. Place pans in oven at 70ºC for one hour.

11.1.8. Remove from oven and weigh as soon as pans are no longer warm (less than 5 minutes). Weigh the pans to the nearest 0.0001 gram.

11.1.9. Calculate percent (%) lipids and GPC loading.

11.2. Bligh & Dyer Option:

11.2.1. Prepare pans: Heat aluminum sample pans in oven at 70° C for a minimum of one hour. Cool pans in a desiccator for a minimum of 30 minutes. Weigh pans to the nearest 0.0001 gram.

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11.2.2. Homogenize tissue in accordance with CAS *SOP Tissue Sample Preparation (MET-TISP).*

11.2.3. Weigh the appropriate amount of tissue into a scintillation vial for samples ≤ 3 grams, into a 40 mL VOA vial for samples 3 - 6 grams, or into a 250 mL beaker for large sample 6 - 30 grams.

11.2.4. Add the appropriate amount of 1:1 Chloroform/Methanol (v/v):

≤ 2 grams, add 10 mL
> 2 - 5 grams, add 25 mL
5 - 10 grams, add 50 mL
10 - 30 grams, add 100 mL

11.2.5. Using a tissumizer, homogenize the sample/solvent mixture for approximately 1 minute or until sample is thoroughly dessimated. Rinse the tissuemizer with solvent. Collect the rinsate with the sample extract. Centrifuge the sample extract at 1000xG for 10 minutes. Transfer extract to a clean VOA vial, leaving the tissue behind. Rinse the tissue portion by adding 1-2 mL of solvent to the tissue. Vortex, centrifuge, and combine with the extract in the VOA vial. Examine the extract to verify that a single phase is present. High water content can result in phase separation. In these cases, add solvent in 1 mL increments until a single phase is achieved.

11.2.6. **Low Volume Extractions**: Back extract by adding 7 mL of sodium sulfate/DI water (25g sulfate/1000mL H2O) to the extract). Vortex extract and centrifuge at 1000xG for 10 minutes. Lipids will be retained in bottom chloroform layer. Pipette the top water layer off the bottom chloroform solvent layer and discard. If colloidal proteinaceous material is observed, add 0.5 mL of sodium sulfate/DI water, vortex, and centrifuge for another 10 minutes. Discard the top water layer.

11.2.7. **High Volume Extractions**: Transfer the extract with rinses into a 250 mL separatory funnel. Back extract 2 times with 100 mL sodium sulfate/DI water, discarding the top water layer each time (lipids will be retained in the bottom layer). Filter the bottom layer through muffled anhydrous sodium sulfate into a KD. Rinse the funnel with 10 - 20 mL of dichloromethane. Concentrate to approximately 5 mL on the S-evap, keeping the temperature ≤ 80°C. The extract may need to be centrifuged or filtered at this point.

11.2.8. Concentrate the extract to approximately 2mL using nitrogen.

11.2.9. Place pans on a hot plate at a setting of 3. Filter the extracts through 3mL syringes into the pans and evaporate to dryness. DO NOT LET EXTRACTS

BOIL. Heat sample pans in oven at 70° C for a minimum of one hour. Cool pans in a desiccator for a minimum of 30 minutes. Weigh pans to the nearest 0.0001 gram and record the weight.

12. QA/QC REQUIREMENTS

12.1. QC Samples Required

12.1.1. Analyze one sample in triplicate per 20 samples.

12.1.2. Analyze one blank, an empty aluminum pan, per twenty samples.

12.2. Acceptance Criteria

12.2.1. RPD for duplicates should be <20%.

12.2.2. Method blank weight should be less than the absolute value of 0.5mg.

12.3. Calculations

12.3.1. Calculate percent (%) lipids

PSEP Option:

$$\%\,Lipids = \frac{\left[\left(Wt_{dish+lipid\,(g)} - Wt_{dish(g)}\right) - Blank\right] \times 5}{Sample\ Wt.(g)} \times 100$$

Bligh & Dyer Option:

$$\%\,Lipids = \frac{\left[\left(Wt_{dish+lipid\,(g)} - Wt_{dish(g)}\right) - Blank\right]}{Sample\ Wt.(g)} \times 100$$

12.3.2. Calculate number of (g) loaded on GPC (5 ml):

$$\frac{\%\ Lipid}{200} \times Sample\ Wt.(g)$$

12.4. Reporting

12.4.1. Fill out the Excel report for % lipids found at R:\SVG\Forms\lipids.

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12.4.2. Samples are weighed to the nearest 0.1mg and report to the nearest 0.1%.

12.4.3. Report results using no more than 3 significant figures.

12.5. Data review

12.5.1. It is the analyst's responsibility to review analytical data to ensure that all quality control requirements have been met for each analytical run. Results for QC analyses are calculated and recorded as specified. All data will be initialed, dated and attached to required data quality worksheet.

12.5.2. The data packet for the sequence is submitted for review by supervisor or designee. The results are transferred to the appropriate report form located in the CAS network directory R:\SVG\WIP.

12.5.3. Refer to the SOP for *Laboratory Data Review Process* for general instructions for data review.

13. CONTINGENCIES FOR HANDLING OUT-OF-CONTROL OR UNACCEPTABLE DATA

Corrective action measures applicable to specific analysis steps are discussed in the applicable section of this (and other applicable) SOP(s). Also, refer to the SOP for *Corrective Action* for correct procedures for identifying and documenting such data. Procedures for applying data qualifiers are described in the SOP for *Report Generation* or in project-specific requirements.

14. METHOD PERFORMANCE

This method was validated through single laboratory studies of accuracy and precision. Refer to the reference method for additional method performance data available.

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15. POLLUTION PREVENTION

It is the laboratory's practice to minimize the amount of solvents and reagents used to perform this method wherever technically sound, feasibly possible, and within method requirements. Standards are prepared in volumes consistent with laboratory use in order to minimize the volume of expired standards to be disposed of. The threat to the environment from solvents and/or reagents used in this method may be minimized when recycled or disposed of properly.

16. WASTE MANAGEMENT

The laboratory will comply with all Federal, State, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions as specified in the laboratory Safety Manual, Sections 6 and 7.

17. TRAINING

17.1. Training outline

17.1.1. Review literature (see references section). Read and understand this SOP. Also review the applicable MSDS for all solvents used. Following these reviews, observe the procedure as performed by an experienced analyst at least three times.

17.1.2. The next training step is to assist in the procedure under the guidance of an experienced analyst. During this period, the analyst is expected to transition from a role of assisting, to performing the procedure with minimal oversight from an experienced analyst.

17.1.3. Training is documented following the SOP *for Documentation of Training*.

17.1.4. NOTE: When the analyst training is documented by the supervisor on internal training documentation forms, the supervisor is acknowledging that the analyst has read and understands this SOP and that adequate training has been given to the analyst to competently perform the analysis independently.

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18. REFERENCES

18.1. USEPA Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Volume 1 - Fish Sampling and Analysis, Third Edition, November, 2000.

18.2. Recommended Protocols for Measuring Selected Environmental Variables in Puget Sound, January 1996 and subsequent chapter revisions.

18.3. Bligh & Dyer, Canadian J. Biochemistry and Physiology, Aug. 1959

18.4. NOAA (National Oceanic and Atmospheric Administration). 1993a. *Sampling and Analytical Methods of the National Status and Trends Program, National Benthic Surveillance and Mussel Watch Projects 1984-1992 Volume 11.Comprehensive Descriptions of Complementary Measurements.* NOAA Technical Memorandum NOS ORCA 71. Coastal Monitoring and Bioeffects Assessment Division, Office of Ocean Resources Conservation and Assessment, National Ocean Service, Silver Spring, MD. July.

18.5. NOAA (National Oceanic and Atmospheric Administration). 1993c. *Sampling and Analytical Methods of the National Status and Trends Program, National Benthic surveillance and Mussel Watch Projects 1984-1992. Volume IV. Comprehensive Descriptions of Trance Organic Analytical Methods.* NOAA Technical Memorandum NOS ORCA 71. Coastal Monitoring and Bioeffects Assessment Division, Office of Ocean Resources Conservation and Assessment, National Ocean Service, Silver Spring, MD. July.

19. CHANGES SINCE THE LAST REVISION

19.1. Changed SOP No from SOC-LIPID to EXT-LIPID
19.2. Updated references to Corporate SOPs through-out the document.
19.3. Changed methylene chloride to dichloromethane.
19.4. Sec 12.2.1 Re-worded to clarify
19.5. Added new NOAA references (18)
19.6. Added standard training section 17 to the SOP.
19.7. Removed reference to MDL study (14)

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STANDARD OPERATING PROCEDURE

CARBON, TOTAL ORGANIC IN SOIL

GEN-ASTM
Revision 7
August 26, 2011

Approved By:

Supervisor	Date
QA Manager	Date
Laboratory Director	Date

COLUMBIA ANALYTICAL SERVICES, INC.
1317 South 13th Avenue
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CARBON, TOTAL ORGANIC IN SOIL

1. SCOPE AND APPLICATION

1.1. This procedure is applicable to the determination of Total Organic Carbon (TOC) using ASTM method D4129-05, modified for soil and sediment matrices (Puget Sound Estuary Program and Lloyd Kahn). Total organic carbon is a measure of the total amount nonvolatile, partially volatile and particulate organic compounds in a sample. Sample should be treated to remove inorganic carbon (carbonates, bicarbonates, free CO_2 etc.), prior to analysis, as these compounds will interfere with true readings.

1.2. This method is applicable to all soils and sediments and most matrices that can be dried and shatter-boxed to a fine powder.

1.3. Results are reported as percent (%) carbon, and the applicable range is the MDL - 100%. The Method Reporting Limit (MRL) for TOC on soils is 0.05%, dry weight basis. Equivalent nomenclature for MRL includes Estimated Quantitation Limit (EQL). Therefore, MRL=EQL. The Method Detection Limit (MDL) has been determined at 0.02%.

2. METHOD SUMMARY

2.1. Samples are combusted in an oxygen atmosphere to convert organic and inorganic forms of carbon to CO_2. The combustion temperature is selected to completely oxidize all carbon forms. The combustion product gases are swept through a barium chromate catalyst/scrubber to ensure that all of the carbon is oxidized to CO_2. Other potentially interfering product gases such as SO_2, SO_3, HX, and NO_x are removed from the gas stream in a series of chemical scrubbers. The CO_2 is then swept to the coulometer where it is detected by automatic, coulometric titration, with coulometric end point indication.

2.2. The coulometer cell is filled with a partially aqueous medium containing ethanolamine and a colorimetric indicator. When a gas stream passes through the solution, CO_2 is quantitatively absorbed. CO_2 reacts with the ethanolamine to form a strong titratable acid which caused the indicator to fade. The titration current automatically turns on and electrically generates base to return the solution to its original color.

3. DEFINITIONS

3.1. Analysis Batch - Samples are analyzed in a set referred to as an analysis batch. The batch begins with calibration/standardization followed by QC analyses and samples. The batch ends when the QC analyses and set of samples has been completed.

3.2. Method Blank - The method blank is an artificial sample (empty boat) designed to monitor introduction of artifacts into the process. The method blank is carried through the entire analytical procedure.

3.3. Laboratory Control Sample (LCS) - A standard of known TOC concentration which is used to ensure that the analysis produces an accurate measurement of TOC in samples analyzed in the batch.

3.4. Matrix Spike/Duplicate Matrix Spike (MS/DMS) Analysis - In the matrix spike analysis, predetermined quantities of target analytes are added to a sample matrix prior to sample preparation and analysis. The purpose of the matrix spike is to evaluate the effects of the sample matrix on the method used for the analysis. Duplicate samples are spiked, and analyzed as a MS/DMS pair. Percent recoveries are calculated for each of the analytes detected. The relative percent difference (RPD) between the duplicate spikes (or samples) is calculated and used to assess analytical precision.

3.5. Laboratory Duplicates (DUP) – Duplicates are additional replicates of samples that are subjected to the same preparation and analytical scheme as the original sample. The relative percent difference (RPD) between the sample and its duplicate is calculated and used to assess analytical precision.

4. INTERFERENCES

4.1. Acidic and other gases, including SO_2, SO_3, H_2S, HCl, HBr, HI, Cl_2, and NO_x can be effectively removed using scrubbers such as KI, Ag_2SO_4, $AgNo_3$, and MnO_2.

4.2. Volatile organics may be lost in the decarbonization process.

5. SAFETY

5.1. All appropriate safety precautions for handling solvents, reagents and samples must be taken when performing this procedure. This includes the use of personnel protective equipment, such as, safety glasses, lab coat and the correct gloves.

5.2. Chemicals, reagents and standards must be handled as described in the CAS safety policies, approved methods and in MSDSs where available. Refer to the CAS Environmental, Health and Safety Manual and the appropriate MSDS prior to beginning this method.

5.3. Hydrochloric and/or Nitric Acid are used in this method. These acids are extremely corrosive and care must be taken while handling them. A face shield should be used while pouring acids. And safety glasses should be worn while working with the solutions. Lab coat and gloves should always be worn while working with these solutions.

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5.4. Disconnect teflon tubing from furnace at check valve whenever system is not in use or when O_2 flow is turned off or furnace temperature is reduced. If the carbon cathode solution should be siphoned through a failed check valve into the magnesium perchlorate scrubber potentially explosive DMSO-perchlorate could be formed.

5.5. Do not attempt to combust large samples of organic or other materials that will react with pure oxygen. Such samples can cause the pyrolysis tube to explode.

6. SAMPLE COLLECTION, CONTAINERS, PRESERVATION AND STORAGE

Samples can be collected in glass or plastic containers. Samples are preserved by storage at 4±2°C. Samples are analyzed within 28 days of collection.

7. APPARATUS AND EQUIPMENT

7.1. Induction furnace, Coulometrics Incorporated.

7.2. Analytical balance, 0.1mg accuracy.

7.3. Desiccator.

7.4. Quartz combustion boats.

7.5. Sample scoop.

7.6. Porcelain dishes.

7.7. Glass ladles and miscellaneous laboratory glassware,

8. STANDARDS, REAGENTS, AND CONSUMABLE MATERIALS

8.1. Reagent grade chemicals shall be used in all tests. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lowering the accuracy of the determination. The preparation for all laboratory prepared reagents and solutions must be documented in a laboratory logbook. Refer to the SOP *Reagent/Standards Login and Tracking (ADM-RTL)* for the complete procedure and documentation requirements.

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8.2. Standards

8.2.1. Urea - 20% carbon. Use 10 µg.

8.2.2. Nutrients in Soil, purchased standard with a known TOC value (typically ERA #542). Use 50 mg for LCS.

8.3. Reagents

8.3.1. Hydrochloric acid, 50% and 10%.

8.3.2. Carbon Cathode Solution. Dimethyl Sulfoxide; DMSO. Purchased from Coulometrics Inc. as a prepared solution. Used for coulometer solution.

8.3.3. Anode Solution. Dimethyl Sulfoxide and potassium iodide. Purchased from Coulometrics Inc. as prepared solution.

8.3.4. Manganese dioxide. Gas scrubber solution.

8.3.5. Potassium Hydroxide. Gas scrubber solution.

8.3.6. Potassium Iodide. Anode chemical.

8.3.7. Magnesium Perchlorate desiccant

9. RESPONSIBILITIES

9.1. It is the responsibility of the analyst to perform the analysis according to this SOP and to complete all documentation required for data review. Analysis and interpretation of the results are performed by personnel in the laboratory who have demonstrated the ability to generate acceptable results utilizing this SOP. This demonstration is in accordance with the training program of the laboratory. Final review and sign-off of the data is performed by the department supervisor/manager or designee.

9.2. It is the responsibility of the department supervisor/manager to document analyst training. Documenting method proficiency, as described in the *SOP for Documentation of Training*, is also the responsibility of the department supervisor/manager.

10. PREVENTIVE MAINTENANCE

10.1. All maintenance activities are recorded in a maintenance logbook kept for each instrument. Pertinent information (serial numbers, instrument I.D., etc.) must be in the logbook. This includes the routine maintenance described in section 10. The entry in the log must include: date of event, the initials of who performed the work, and a reference to analytical control.

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Maintenance is performed as follows:

Maintenance Item	Frequency
Cell	Clean daily with methanol and water to clean frit
Mg Perchlorate Scrubber	change daily
KOH Scrubber	change monthly
NOX scrubber	change as needed
Repack Precombustion Column	as needed
Repack Combustion Column	as needed

11. PROCEDURE

11.1. Sample Preparation.

11.1.1. Turn furnace on to #5 (≈1000°C). Allow furnace to warm-up for about 1/2 hours. Turn on oxygen to ≈ 5 psi and 75 to 125 ml/min at flowmeter.

11.1.2. Clean quartz boats. Scrape out old sample and rinse boats with DI water. Place boats in crucible and muffle for at least 10-15 minutes. Remove boats and place in desiccator until ready for use.

11.1.3. Samples should be dried at 70°C and homogenized prior to analysis. Homogenization of dried solid sample should include grinding with a mortar and pestle or shatter box. A shatter box should be used with a larger sample size (i.e. 20+ grams) if the sample exhibits a high degree of heterogeneity. Samples should be ground to a fine, homogenous, powder.

11.1.4. Ground samples must be stored in individual sealed vials. In addition, sample vials analyzed under PSEP methodology must be stored in a desiccator prior to sample analysis.

11.1.5. As a rule, the darker (or closer to black) a sample is, the more carbon it contains. Place a small portion of sample on a watch glass. Add 1 drop of 10% HCl. Watch for effervescence or bubbling. If bubbles are present, the sample contains inorganic carbon (CO_3). If sample bubbles, reduce sample size to prevent sample from bubbling out of boat. If sample is dark, wood product or sludge reduce sample volume to 5 $\rightarrow$ 10mg. Normal sample volume = 50mg. After boats are loaded with sample add 1 to 2 drops 10% HCl. Place boats in 70°C oven to dry. If samples bubbled when acid was added, add 1 to 2 drops more acid and dry at 70°C.

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Continue acidifying and drying until samples no longer bubble. Place samples in desiccator until ready for analysis.

11.2. Apparatus Preparation.

11.2.1. Fill cell with carbon cathode solution to 100 → 125 ml, drop in stir bar. Place cell top on snug.

11.2.2. Cover bottom of anode cell with KI. About 2 small scoops.

11.2.3. Add carbon anode solution to cell such that when anode is inserted in the anode cell, the anode solution level is the same as the cathode solution level.

11.2.4. Place cell in coulometer cell holder.

11.2.5. Turn on detector lamp and stir plate. (Power on)

11.2.6. Turn adjust knob to 122 (all the way to the right) then turn back down to 100. Rotate cell until maximum transmittance is obtained.

11.2.7. With oxygen bubbling to cell and maximum transmittance obtained, turn on the current to the anode and cathode. The carbon cathode solution will begin to titrate to a blue color.

11.2.8. Change Magnesium Perchlorate desiccant daily.

11.2.9. The instrument is now ready to run.

11.3. Calibration and Standardization.

11.3.1. Burn both ladles for five minutes each to remove any residual TOC.

11.3.2. Establish baseline.

11.3.2.1. After placing ladles in sample inlet, allow system to purge for 1 minute.

11.3.2.2. Burn three boats empty five minutes each. The average of the three runs is the baseline.

11.4. Analysis.

11.4.1. Place one platinum or quartz boat in a ladle. Place the ladle in the sample inlet and purge for 1 minute. Simultaneously insert the sample into the furnace, press the reset button on the coulometer and start the timer for five minutes.

11.4.2. After five minutes, obtain a reading from the instrument. Remove the ladle from the furnace. (Occasionally, a high sample may require longer than 5 minutes to complete the titration).

11.4.3. Load the other ladle with the next platinum (or quartz) boat. Remove the ladle in use from the inlet port and insert the next ladle.

11.4.4. Repeat steps 11.4.1 through 11.4.3 until all samples are analyzed.

12. QA/QC REQUIREMENTS

12.1. Initial Precision and Recovery Validation

The precision and accuracy of the procedure must be validated before analysis of samples begins, or whenever significant changes to the procedures have been made. To do this, four LCS's are prepared and analyzed. The RSD should be <20% and average recovery must be 85-115%.

12.2. Method Detection Limits and Method Reporting Limits

12.2.1. A method detection limit (MDL) study must be undertaken before analysis of samples can begin. To establish detection limits that are precise and accurate, the analyst must perform the following procedure. Analyze a minimum of seven spiked blank replicates at a level near the MRL. Follow the procedures starting in Section 11 to analyze the samples. Refer to the CAS *SOP Performing Method Detection Limit Studies and Establishing Limits of Detection and Quantification (ADM-MDL).* The MDL study must be verified annually.

12.2.2. Calculate the average concentration found (x) in the *sample concentration*, and the standard deviation of the concentrations for each analyte. Calculate the MDL for each analyte using the correct T value for the number of replicates.

12.2.3. Limits of Quantification (LOQ)

12.2.3.1.The laboratory establishes a LOQ for each analyte as the lowest reliable laboratory reporting concentration or in most cases the lowest point in the calibration curve which is less than or equal to the desired regulatory action levels, based on the stated project requirements. Analysis of a standard or extract prepared at the lowest point calibration standard provides confirmation of the established sensitivity of the method. The LOQ recoveries should be within 75-125% of the true values to verify the data reporting limit. Refer to the CAS SOP *Performing Method Detection Limit Studies and Establishing Limits of Detection and Quantification (ADM-MDL)*

12.2.4. The Method Reporting Limits (MRLs) used at CAS are the routinely reported lower limits of quantitation which take into account day-to-day fluctuations in instrument sensitivity as well as other factors. These MRLs are the levels to which CAS routinely reports results in order to minimize false positive or false negative results. The MRL is normally two to ten times the method detection limit.

12.3. Ongoing QC Samples required are described in the CAS-Kelso Quality Assurance Manual and in the SOP for Sample Batches. Additional QC Samples may be required in project specific quality assurance plans (QAPP). For example projects managed under the DoD ELAP must follow requirements defined in the DoD *Quality Systems Manual for Environmental Laboratories.* General QA requirements for DoD QSM are defined in the laboratory SOP, Department *of Defense Projects – Laboratory Practices and Project Management (ADM-DOD).*

12.4. The QC criteria discussed in the following sections are summarized in Table 1.

12.4.1. LCS - An LCS must be analyzed with each batch of 20 or fewer samples. Analyze 50mg of the purchased standard (see 8.1.2) is used. The acceptance criteria for the LCS are listed in Table 1.

12.4.2. Method Blank - Burn one empty boat per batch of 20 or fewer samples. Method Blank must be <0.05% carbon.

12.4.3. CCV (Continuing Calibration Verification) - A CCV must be analyzed every tenth analysis. Analyze ~10mg urea. The CCV must be 18.0% - 22.0% carbon.

12.4.4. CCB (Continuing Calibration Blank) - A CCB must be analyzed following every CCV.

12.4.5. Sample duplicate – ASTM D 4129: One duplicate sample per batch of 20 or fewer samples must be analyzed in duplicate. TOC analysis by PSEP methodology requires one sample to be analyzed in triplicate per batch of 20 or fewer samples. Samples analyzed under Loyd Kahn methodology must all be analyzed in duplicate. All duplicates and triplicates, regardless of the method cited, should be within 20% RPD, if > five times the MRL.

12.4.6. Matrix Spike - One spike must be analyzed with each batch of 20 or fewer samples. The acidified sample will be spiked with a known amount of urea.

12.4.7. See Table 1 for a summary of acceptance criteria and corrective actions.

13. DATA REDUCTION AND REPORTING

13.1. Calculate % carbon as follows:

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$$\%Carbon = \frac{(Gross\ reading\ -\ baseline\ \mu g)(0.1)}{mg\ sample\ analyzed}$$

13.1.1. Total organic carbon is reported as % carbon, normally on a dry weight basis. Results may be reported on an as received basis.

13.2. For duplicate analyses, calculate relative percent difference as follows:

$$RPD = \frac{S_1 - S_2}{Avg} * 100$$

where S1 = Sample with higher value
S2 = Sample with lower value
Avg = Average of the two sample values

13.3. Calculate percent recovery as follows:

$$\%R = \frac{X - X1}{TV} \times 100$$

where X = Concentration of the analyte recovered
X1 = Concentration of unspiked analyte
TV = True value of amount spiked

13.4. Data Review and Assessment

13.4.1. Following primary data interpretation and calculations, all data is reviewed by a secondary analyst. Following generation of the report, the report is also reviewed. Refer to the *SOP for Laboratory Data Review Process (ADM-DREV)* for details. The person responsible for final review of the data report and/or data package should assess the overall validity and quality of the results and provide any appropriate comments and information to the Project Chemist to inclusion in the report narrative. All data will be initialed, dated and attached to required data quality worksheet.

13.5. Reporting

13.5.1. Refer to the SOP for *Data Reporting and Report Generation* (ADM-RG) for reporting guidelines.

13.5.2. The analyst enters data directly into CASLIMS templates. An Analytical Results Summary is generated for that analytical batch showing all QC and sample results. After primary and secondary review, final reports are generated in CASLIMS by compiling the SMO login, sample prep database, instrument date, and client-specified

report requirements (when specified). The forms generated may be CAS standard reports, DOD, or client-specific reports. The compiled data from LIMS is also used to create EDDs.

13.5.3. As an alternative, reports are generated using Excel© templates located in R:\WET. The analyst should choose the appropriate form and QC pages to correspond to required tier level and deliverables requirements. The results are then transferred, by hand or electronically, to the templates.

14. CONTENGENCIES FOR HANDLING OUT-OF-CONTROL OR UNACCEPTABLE DATA

Corrective action measures applicable to specific analysis steps are discussed in the applicable section of this (and other applicable) SOP(s). Also, refer to the SOP for Nonconformity and Corrective Action for correct procedures for identifying and documenting such data. Procedures for applying data qualifiers are described in the SOP for Report Generation or in project-specific requirements.

15. METHOD PERFORMANCE

15.1. This method is validated through single laboratory studies of accuracy and precision. Refer to the reference method for additional method performance data available. The method detection limit (MDL) is established using the procedure described in the SOP for *Performing Method Detection Limit Studies and Establishing Limits of Detection and Quantification (ADM-MDL).*

15.2. Method Reporting Limits are established for this method based on MDL studies and as specified in the CAS Quality Assurance Manual.

16. POLLUTION PREVENTION

It is the laboratory's practice to minimize the amount of solvents, acids and reagent used to perform this method wherever feasible. Standards are prepared in volumes consistent with methodology and only the amount needed for routine laboratory use is kept on site. The threat to the environment from solvent and reagents used in this method can be minimized when recycled or disposed of properly.

17. WASTE MANAGEMENT

17.1. The laboratory will comply with all Federal, State and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions as specified in the CAS EH&S Manual.

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17.2. This method uses acid. Waste acid is hazardous to the sewer system and to the environment. All acid waste must be neutralized to a pH of 2.5-12 prior to disposal down the drain. The neutralization step is considered hazardous waste treatment and must be documented on the treatment by generator record. See the CAS EH&S Manual for details.

17.3. This method uses a base. Waste base is hazardous to the sewer system and to the environment. All waste must be neutralized to a pH of 2.5-12 prior to disposal down the drain. The neutralization step is considered hazardous waste treatment and must be documented on the treatment by generator record. See the CAS EH&S Manual for details.

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18. REFERENCES

18.1. Coulometrics Inc. Instruction Manual, Model 5020.

18.2. Total Organic Carbon(TOC), Conventional Sediment Variables, Puget Sound Estuary Program, March 1986.

18.3. Determination of Total Organic Carbon in Sediment, Lloyd and Kahn, U.S.E.P.A Region II, July 1998.

18.4. ASTM Method D4129-05.

19. CHANGES SINCE THE LAST REVISION

19.1. Removed reference to method 9060, a IR method
19.2. Sec 1.3 removed PQL
19.3. Sec 3.4 and 3.5 are new
19.4. Sec 8.1 is new
19.5. Sec 11.1.3 and 11.1.4 is new
19.6. Sec 12.2.1 updated sop reference and verification requirements
19.7. Sec 12.2 revised to reflect lab QA systems requirements
19.8. Sec 13.4 and 13.5 updated to reflect lab QA systems requirements
19.9. Table 1 updated

TABLE 1
Summary of Corrective Actions

Method Reference	Control	Specification and Frequency	Acceptance Criteria	Corrective Action
ASTM D4129 PSEP Loyd Kahn	CCV	Verify calibration by analyzing prior to samples, after every 10 analysis and after the last sample	±10%	Re-analyze all samples affected.
ASTM D4129 PSEP Loyd Kahn	LCS	Include with each analysis batch (up to 20 samples)	74-118%	Re-analyze all samples affected.
ASTM D4129 PSEP Loyd Kahn	Method Blank	Include with each analysis batch (up to 20 samples)	< 0.05%	If target exceeds 0.05%, clean boats and re-analyze.
ASTM D4129 PSEP Loyd Kahn	Matrix Spike	Include with each analysis batch (up to 20 samples)	69-123%	Evaluate data to determine if the there is a matrix effect or analytical error
ASTM D4129	Sample Duplicates	Include with each analysis batch (up to 20 samples)	≤ 20 % RPD	Re-homogenize and re-analyze if result is > 5 X the MRL
PSEP	Sample Triplicate	Include with each analysis batch (up to 20 samples)	≤ 20 % RSD	Re-homogenize and re-analyze if result is > 5 X the MRL
Loyd Kahn	Sample Duplicates	All samples in each analysis batch	≤ 20 % RPD	Re-homogenize and re-analyze if result is > 5 X the MRL

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APPENDIX I

BENCHSHEETS

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Columbia Analytical Services, Inc.

Service Request #: _____ Method: ASTM D4129-05/EPA Lkahn/ PSEP (Combustion/Coulometric)

Analysis For: Total Organic Carbon (TOC) Matrix: Soil / Dry Weight Basis

Sample Number	mg Sample Injected	Sample Reading, µg C	Baseline Reading, µg C	Net µg C	% Carbon	% Carbon Reported
CCV-1						
CCB-1	50.0		0.0	#VALUE!	#VALUE!	#VALUE!
LCS-1						
MB-1	50.0		0.0	#VALUE!	#VALUE!	#VALUE!
CCV-2						
CCB-2	50.0		0.0	#VALUE!	#VALUE!	#VALUE!
CCV-3						
CCB-3	50.0		0.0	#VALUE!	#VALUE!	#VALUE!

Revision:1 R:\WET\ANALYSES\TOC\TEMPLATE\Copy of TOCsoil_LIMS

Acid Purge Time: 1 minute **Reading Time:** 5 minutes **TOC % =** $\frac{\text{(Net Reading)(µg 0.1)}}{\text{mg Sample Injected}}$

CCV : Urea EMD (lot #) ID#: TV = 20.0%C

CCV1 = #VALUE! CCV2 = #VALUE! CCV3= #VALUE!

LCS: ERA Cat#: 542 Lot#: ID#: TV = 0.28%C %REC=

Comments :

Kms =mg x 20 /= % REC= RSD= x=

Kmsd =mg x 20 / = % REC=

K=mgx20/=

date time

Analyzed By:	Date:
Reveiwed By:	Date:

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STANDARD OPERATING PROCEDURE

SULFIDES, ACIDS VOLATILE

GEN-AVS
Revision 6

Effective date: February 6, 2012

Approved By:

Supervisor	1/6/12 Date
QA Manager	1/6/12 Date
Laboratory Director	1/6/12 Date

COLUMBIA ANALYTICAL SERVICES, INC.
1317 South 13th Avenue
Kelso, Washington 98626

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DOCUMENT CONTROL	
NUMBER:	
Initials:	Date:

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SULFIDES, ACIDS VOLATILE

1. SCOPE AND APPLICATION

1.1. This procedure describes the determination of acid volatile sulfide (AVS) in soil, sediment, and other solids. As a precipitant of toxic heavy metals, sulfide is important in controlling the bioavailability of metals in sediments. The procedure can be used to isolate those metals, referred to as Simultaneously Extracted Metals (SEM), solubilized during the acidification step. Analysis for these metals can then be done using the applicable metals determinative procedure.

1.2. Research has established that the relative amounts of SEM and AVS are important in the prediction of potential metal bioavailability; if the molar ratio of SEM for bivalent metals to AVS exceeds one, the toxic heavy metals in that sample are potentially bioavailable. This method uses the same conditions for release of both sulfide and metal from the sediment and thus provides a useful means of assessing the amount of metal associated with sulfide.

1.3. This procedure also includes steps for determination of Chromium Reducible Sulfur.

1.4. This method is capable of determining sulfides in the range of 0.5 mg/Kg to 30,000 mg/Kg dry weight. The Method Reporting Limit (MRL) 0.5 mg/Kg. Method detection limits are 0.05 mg/Kg in the sample.

2. METHOD SUMMARY

2.1. The AVS in the sample is first converted to hydrogen sulfide (H_2S) by acidification with hydrochloric acid at room temperature. The H_2S is then purged from the sample and trapped in aqueous solution. The amount of sulfide that has been trapped is then determined. If SEM is to be determined, a portion of the aqueous solution is used to perform selected metals analyses.

2.2. The H_2S released by acidifying the sample is quantified using a colorimetric procedure. In the colorimetric method, the H_2S is trapped in sodium hydroxide. The sulfide reacts with N-N dimethyl-p-phenylenediamine to form methylene blue that is measured. This procedure is capable of determining AVS concentrations as low as 0.5 mg/Kg dry weight of sediment. By appropriate sample dilution, the maximum concentration of AVS which can be determined is at least 30,000 mg/Kg dry sediment.

3. DEFINITIONS

3.1. **Batch** - A batch of samples is a group of environmental samples that are prepared and/or analyzed together as a unit with the same process and personnel using the same lot(s) of reagents. It is the basic unit for analytical quality control.

3.1.1. Preparation Batch - A preparation batch is composed of one to twenty field samples, all of the same matrix, meeting the criteria in Section 3.3 and with a maximum time between the start of processing of the first and last samples in the batch to be 24 hours.

3.2. **Sample**

3.2.1. Field Sample - An environmental sample collected and delivered to the laboratory for analysis; a.k.a., client's sample.

3.2.2. Laboratory Sample - A representative portion, aliquot, or subsample of a field sample upon which laboratory analyses are made and results generated.

3.3. **Quality System Matrix** - The *matrix* of an environmental sample is distinguished by its physical and/or chemical state and by the program for which the results are intended. The following sections describe the matrix distinctions. These matrices shall be used for purpose of batch and quality control requirements.

3.4. Matrix Spike/Duplicate Matrix Spike (MS/DMS) Analysis - In the matrix spike analysis, predetermined quantities of target analytes are added to a sample matrix prior to sample preparation and analysis. The purpose of the matrix spike is to evaluate the effects of the sample matrix on the method used for the analysis. Duplicate samples are spiked, and analyzed as a MS/DMS pair. Percent recoveries are calculated for each of the analytes detected. The relative percent difference (RPD) between the duplicate spikes (or samples) is calculated and used to assess analytical precision. The concentration of the spike should be at the mid point of the calibration range or at levels specified by a project analysis plan.

3.5. Laboratory Duplicates (DUP) – Duplicates are additional replicates of samples that are subjected to the same preparation and analytical scheme as the original sample. The relative percent difference (RPD) between the sample and its duplicate is calculated and used to assess analytical precision.

3.6. Method Blank (MB) - The method blank is an artificial sample composed of analyte-free water or solid matrix and is designed to monitor the introduction of artifacts into the analytical process. The method blank is carried through the entire analytical procedure.

3.7. Laboratory Control Samples (LCS) – The LCS is an aliquot of analyte free water or analyte free solid to which known amounts target analytes are added. The LCS is prepared and

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analyzed in exactly the same manner as the samples. The percent recovery is compared to established limits and assists in determining whether the batch is in control.

3.8. Acid Volatile Sulfide (AVS) - AVS is defined as sulfides that form hydrogen sulfide under the conditions of this test. This includes amorphous, moderately crystalline monosulfides, and other sulfides.

3.9. Method Detection Limit (MDL) - The minimum concentration of an analyte that can be measured and reported with 99% confidence that the analyte concentration is greater than zero. The MDL is determined as described in the SOP for The Determination of Method Detection Limits.

3.10. Stock Standard Solution - A concentrated solution of the analyte prepared in the laboratory using assayed reference compounds or purchased from a reputable commercial source.

3.11. Calibration Standards - Solutions prepared from the stock standard solution that is used to calibrate the method response with respect to analyte concentration.

4. INTERFERENCES

4.1. Contact with oxygen must be avoided in all stages from sampling to analysis. Consequently, the samples and standards should be protected from air from the time of sampling through the analytical procedure.

4.2. Carbonation is an interference that can occur when acid is added to the sample. The carbonation can push the sulfide into the B tower or outside it. To prevent this, acid should be added to the sample slowly and/or the sample weight reduced.

5. SAFETY

5.1. All appropriate safety precautions for handling solvents, reagents and samples must be taken when performing this procedure. This includes the use of personnel protective equipment, such as, safety glasses, lab coat and the correct gloves.

5.2. Chemicals, reagents and standards must be handled as described in the CAS safety policies, approved methods and in MSDSs where available. Refer to the CAS Environmental, Health and Safety Manual and the appropriate MSDS prior to beginning this method.

5.3. Sodium Hydroxide (NaOH) is a strong caustic and a severe health and contact hazard. Use nitrile or latex gloves while handling pellets or preparing solutions.

5.4. Hydrochloric and/or Nitric Acid are used in this method. These acids are extremely corrosive and care must be taken while handling them. A face shield should be used while

pouring acids. And safety glasses should be worn while working with the solutions. Lab coat and gloves should always be worn while working with these solutions.

5.5. Hydrogen sulfide is a highly poisonous, gaseous compound having a characteristic odor of rotten eggs. It is detectable in air by humans at a concentration of approximately 0.002 ppm. Handling of acid samples should be performed in a hood or well ventilated area. If a high concentration of hydrogen sulfide is detected in the air by the laboratory staff, sample handling procedures must be corrected. Exposure to H_2S in air must not exceed guidelines or regulations. The OSHA Permissible Exposure Limit (PEL) is 50ppm.

5.6. If samples originate from a highly contaminated area, appropriate sample handling procedures to minimize worker exposure must be followed.

6. SAMPLE COLLECTION, PRESERVATION AND STORAGE

6.1. Sulfide ion is unstable in the presence of oxygen. Protect sediment samples from exposure to oxygen during sample collection and storage.

6.2. During storage, sulfide can be formed or lost due to biological activity, and sulfide can be lost by volatilization or oxidation. Metal speciation can change as a result of changes in sulfide concentration and as a result of other changes in the sample.

6.3. Samples should be collected in wide mouth jars with a minimum of air space above the sediment. If possible, the headspace should be filled with oxygen free nitrogen or argon. The jar lids must have Teflon or polyethylene liners.

6.4. Samples should be cooled to 4 ± 2 °C as soon as possible after collection. Samples maintained at 4 ± 2 °C have been found to have no significant loss of AVS for storage periods up to 2 weeks. Holding time for samples should not exceed 14 days.

7. APPARATUS AND EQUIPMENT

7.1. Glassware

7.1.1. AVS evolution and H_2S trapping - The Midi is typically used.

7.1.1.1. Midi setup: For each analytical train, use one boiling tube attached to an inlet adapter that is topped with a 50 ml dropping funnel. This apparatus is connected to a fritted bubbler (called the “A” tower) which is then connected by tubing to the “B” tower. The boiling tube contains the sediment and acid is introduced to it by the 50 ml dropping funnel. Because sulfide may react with tubing and other surfaces, minimum lengths of tubing should be used as sleeves to connect the glass tubing.

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7.1.1.2. Macro setup: For each analytical train, one 500 ml round bottom flask with a septum 250 ml scrubber towers with impingers with non-fritted outlets. The round bottom flask contains the sediment and acid is introduced to it by a syringe inserted through the septum. The flasks are connected by tubing. Because sulfide may react with tubing and other surfaces, minimum lengths of tubing should be used as sleeves to connect the glass tubing. The analyst should pay particular attention to the recovery of sulfide from standards in evaluating the apparatus.

7.1.1.3. The analyst should pay particular attention to the recovery of sulfide from standards in evaluating the apparatus used.

7.1.1.4. In all cases, the inlets are below the liquid level and the outlets are above the liquid levels. The apparatus is assembled as shown in Figure 1, and more than one analytical train can be connected to a single cylinder of nitrogen or argon if flow controllers are installed in the line.

7.2. Assorted calibrated pipettes and volumetric flasks.

7.3. Evergreen disposable tubes, 50 mL. Check tubes for accuracy on a per batch basis by filling a tube to the 50 mL mark and measuring the water's mass. The measured mass must be accurate to ± 3%, if not obtain a new lot of tubes and retest. Refer to the SOP for *Checking Volumetric Labware (ADM-VOLWARE)*, for detailed instructions on performing the accuracy test.

7.4. Analytical balance - capable of weighing to 0.0001 g.

7.5. Magnetic stirrer, thermally insulated and Teflon-coated stirring bar.

7.6. Spectrophotometer - Capable of measuring absorbance at 670 nm.

7.7. Spectrophotometer cells, 1mm.

8. STANDARDS, REAGENTS AND CONSUMABLE MATERIALS

8.1. Reagent grade chemicals shall be used in all tests. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lowering the accuracy of the determination. The preparation for all laboratory prepared reagents and solutions must be documented in a laboratory logbook. Refer to the SOP *Reagent/Standards Login and Tracking (ADM-RTL)* for the complete procedure and documentation requirements.

8.2. All water and reagents used in this method must be free of dissolved oxygen and sulfide. Prepare reagents fresh for each batch and use deaerated, deionized water by removing dissolved oxygen from the deionized water by vigorously bubbling with oxygen-free

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nitrogen or argon for approximately one hour. Deaerate reagents immediately before use by deaerating with oxygen-free nitrogen or argon.

8.3. Sulfide stock standard solution, approximately 0.05M or 50 µmoles/ml.

8.3.1. Weigh about 5.25 gram of $Na_2S \cdot 9H_2O$ and dissolve it in 250 ml of 0.5N NaOH. Store in a brown bottle. To prevent air oxidation, the sulfide solution should be maintained under oxygen-free nitrogen or argon. This solution must be standardized daily against thiosulfate to quantitatively determine sulfide concentration. Provided that the solution is standardized daily, at the time of use, the sulfide stock solution may be used for up to six months.

8.3.2. Standardize against thiosulfate solution.

8.3.2.1. Pipette 11.0 ml of 0.025N standard iodine solution into each of two 125-ml Erlenmeyer flask or beakers.

8.3.2.2. Pipette 1.00 ml of sulfide stock standard solution into one flask. Pipette 2.00 ml of deionized water, as a laboratory reagent blank, into the other flask.

8.3.2.3. Add 5.00mL of 6M HCl into each flask, swirl slightly.

8.3.2.4. Titrate each with 0.025N thiosulfate until the yellow iodine color fades to a pale straw. Just before all the iodine has been titrated, add starch indicator dropwise to form a pale blue color. Continue the titration with the thiosulfate. The end point is reached when the blue color first disappears.

8.3.2.5. Calculate the sulfide concentration as follows:

$$Sulfide\,(mg/L) = \frac{\left[\left(mL\ of\ I_2\ x\ N\ of\ I_2\right) - \left(mL\ of\ titrant\ x\ N\ of\ titrant\right)\right] x\, 16030}{Volume\ of\ std}$$

8.4. Iodine Solution (approximately 0.025N)

8.4.1. Dissolve 25 g potassium iodine, KI, in 700 ml of reagent water in a 1 liter volumetric flask. Add 3.2 g iodine, I_2. and allow to dissolve. Add 2 ml of 6N HCl and dilute to 1.0L with reagent water. Standardize against sodium thiosulfate as follows.

8.4.2. Titrate until amber color fades to yellow. Add starch indicator solution. Continue titration drop by drop until the blue color disappears.

8.4.3. Run in duplicate

8.4.4. Calculate the normality as follows:

$$\frac{mls\ S_2O_3\ x\ Normality\ of\ S_2O_3 (0.025N)}{mls\ Iodine\ titrated\ (5\ mls)}$$

8.5. Standard sodium thiosulfate solution (0.025N): Dissolve 6.205 ± 0.005 g $Na_2S_2O_3 \cdot 5H_2O$ in 500 ml reagent water. Add 18 ml of 0.5N NaOH, and dilute to 1 liter.

8.6. Starch indicator - Dissolve 1.0 gram soluble starch in 100 ml boiling deionized water.

8.7. Sulfide working standards - Prepare sulfide working standards using the sulfide stock standard solution in Section 8.3.1. The concentrations of the following standards will depend on the exact concentration of the sulfide stock standard determined in Section 8.3.2.5.

Prepare the sulfide working standard by diluting 1.00mL of sulfide stock standard to 100mL 0.5N NaOH. This solution contains 28.0 mg sulfide/L, if the concentration of the sulfide stock standard is exactly 2800 mg/L. This working solution must be prepared daily from standardized stock solution.

8.8. AVS Evolution reagents

8.8.1. Hydrochloric acid 6M - Dilute 500 ml of concentrated hydrochloric acid to 1.0 L de-aerated reagent water.

8.8.2. Nitrogen gas, oxygen free, with regulator and flow controller.

8.8.3. Plastic hypodermic syringe, 30 ml, and needle.

8.9. Colorimetric reagents

8.9.1. Sodium Hydroxide solution, 0.5N - Dissolve 20 g sodium hydroxide per 1000 ml reagent water. Generally make 4.0 L at one time. (80 g NaOH to 4.0 L)

8.9.2. Mixed diamine reagent, MDR

8.9.2.1. Component A - Add 1320 ml concentrated sulfuric acid to 680 ml of reagent water after solution cools, dissolve 4.50 g N-N-dimethyl-p-phenylenediamine oxalate in it.

8.9.2.2. Component B - Dissolve 10.87 g ferric chloride hexahydrate ($FeCl_3 \cdot 6\ H_2O$) in 200 ml concentrated hydrochloric acid and dilute to 400 ml with reagent water.

8.9.2.3. Mixed diamine reagent, MDR – Slowly mix 2 L component A and 400 mL component B the components A and B (prepared above) and allow to cool.

8.10. Sulfuric acid solution, 1.0M – Dilute 56 mL concentrated H_2SO_4 to 1L reagent water.

8.11. Reagent required for Chromium Reducible Sulfur (Fossing & Jorgensen)

8.11.1. Ethanol, purchased commercially at ≥95% purity.

8.11.2. Concentrated hydrochloric acid (HCl)

8.11.3. Hydrochloric acid, 1N, purchased.

8.11.4. Cr^{+2} solution: Prepare from Cr^{+3} by dissolving 133.2g of Chromium (III) chloride hexahydrate in 500mL of 0.5N HCl. The Cr^{+3} solution is poured over 1N HCl washed mossy zinc in a nitrogen glove box and let sit until the solution turns a deep blue color. At this time the solution can be filtered into glass jars and nitrogen capped until ready to use. The solution will remain blue for a number of days. Remake standard if color fades or after one month, which ever comes first.

9. RESPONSIBILITIES

9.1. It is the responsibility of the analyst to perform the analysis according to this SOP and to complete all documentation required for data review. Analysis and interpretation of the results are performed by personnel in the laboratory who have demonstrated the ability to generate acceptable results utilizing this SOP. This demonstration is in accordance with the training program of the laboratory. Final review and sign-off of the data is performed by the department supervisor/manager or designee.

9.2. It is the responsibility of the department supervisor/manager to document analyst training. Documenting method proficiency, as described in the SOP for Documentation of Technical Personnel Training is also the responsibility of the department supervisor/manager.

10. PREVENTIVE MAINTENANCE

10.1. No specific maintenance steps are needed other than normal cleaning and inspection of apparatus.

10.2. Wipe down the Midi unit after use to remove any residual acid.

11. PROCEDURE

Note: Refer to Appendix A if Chromium Reducible Sulfur is to be determined.

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11.1. Macro distillation procedure

11.1.1. Place two neck flasks on stir plates and clamp into place.

11.1.2. Add one stir bar and 100 ml of reagent water to each flask. The amount of water added and the water present in the wet sediment should not exceed 120 mls.

11.1.3. Add 80 mls of 0.5N NaOH to each of the two towers for each flask. Label the first tower A, the second tower B.

11.1.4. Place septum stopper and impinger in two-neck flask. Place tower scrubbers in towers and connect the glassware with the tubing.

11.1.5. Purge the system with N_2 gas for approximately 10 minutes.

11.1.6. Weigh 10 g of wet sediment onto a 2x2 inch piece of parafilm. Remove the septum stopper and place the parafilm with sample into the flask. Do not rinse sample into flask. Purge the system for another 10 minutes.

Stop the flow of gas. Using the 30 ml hypodermic syringe, add 20 ml of 6M HCl to the flask via septum. Bubble nitrogen through the system for one hour while constantly stirring. For the LCS and spike, add the appropriate amount of sulfide standard before adding the acid.

11.1.7. Calibration and Standardization

11.1.7.1. Prepare a sulfide curve as follows:

11.1.7.1.1. Add 80 mls of 0.5N NaOH to each of 7 - 100 ml volumetric flask.

11.1.7.1.2. Using working standard A, add 0.0 ml, 1.0 ml, 2.0 ml, 1.0 ml, 3.0 ml, 4.0 ml and 5.0 ml to flask. Bring to 100 mL final volume using 0.5 N NaOH.

11.1.7.1.3. Calculate concentration of standards as follows:

$$\frac{\textit{(mls STD added) x (Conc. Of STD)}}{\textit{Final volume (100ml)}} = \text{\_\_\_\_}\textit{mg/L}$$

Example: $\frac{4.0\text{ml} \times 28.0\text{ mg/L}}{100\text{ml}} = 1.12\text{ mg/L}$

11.1.7.1.4. Use the 0 ml STD for the CCB and the 4 ml STD for the CCV.

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11.1.8. After one hour has elapsed, remove the scrubber from tower B and transfer to a 250 ml plastic container. Repeat for tower A. Leave N_2 gas flowing through scrubbers so that the NaOH does not go back up into the scrubber.

11.1.9. Disconnect tubing from flow meter at the flask impinger and then turn off the N_2 at the flow meter. This prevents liquid from backing up into the flow meter and destroying it.

11.1.10. Proceed to section 11.3.

11.2. Midi distillation procedure

11.2.1. Soak the scrubbers in 10% nitric acid and then rinse scrubbers with 10% nitric acid followed by a rinse with deionized water.

11.2.2. Place analytical train, consisting of the boiling tube, inlet adapter and 50 ml dropping funnel, into the midi apparatus.

11.2.3. Add 40mL of 0.5N NaOH to each bubbler vessel and clamp scrubber to bubbler vessel. Connect scrubber, with bubbler vessel attached, to the analytical train.

11.2.4. Purge the system for approximately 10 minutes.

11.2.5. Add 10.0g of sample (or less depending on expected sulfide concentration) to the boiling tube. Rinse sample to the bottom of the boiling tube with 40mL of reagent water. Make sure no sample remains above the reagent water level. Clamp the impinger to the boiling tube.

11.2.6. Calibration and Standardization

11.2.6.1.Prepare a sulfide curve as follows:

11.2.6.1.1.Add 35 mls of 0.5N NaOH to each of 7- 50 ml centrifuge tubes.

11.2.6.1.2.Using working standard A, add 0 ml, 0.05 ml, 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml and 2.5 ml to each tube. Bring to 50 mL final volume using 0.5 N NaOH.

11.2.6.1.3.Calculate concentration of standards as follows:

$$\frac{(\text{mls STD added}) \times (\text{Conc. Of STD})}{\text{Final volume (40ml)}} = \text{\_\_\_\_mg/L}$$

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Example: $\frac{2.5ml \times 28.0\ mg/L}{40ml} = 1.75\ mg/L$

11.2.6.1.4. Use the 0 ml STD for the CCB and the 1.5 ml STD Check for the CCV.

11.2.7. Purge system for 10 minutes.

11.2.8. Connect scrubber tube with a disposable pipet to scrubber extension. Place the pipet into 50mL centrifuge tube filled with 40mL of 0.5N NaOH. (These are the "B" towers.)

11.2.9. Purge for 10 minutes.

11.2.10. Add the LCS and the Spike(s).

11.2.10.1. For aqueous blank spikes, add 2mL of the sulfide working standard to 50mL of purged reagent water.

11.2.10.2. For soils and solids, weigh 10g of analyte-free glass beads and add 50mL of reagent water. Purge for 10 minutes, then add 2mL of the sulfide working standard.

11.2.10.3. For matrix spikes, spike the sample with 1mL of the sulfide stock standard.

11.2.11. Add 10.0 mL of 6N HCl to each boiling tube through the 50 ml dropping funnel.

11.2.12. Let system react for 1 hour.

11.2.13. Place scrubber solution from bubbler vessels into separate containers. Before screwing on the cap, cover each solution with nitrogen. The same procedure is used for B towers except that the scrubber solution is left in the original centrifuge tube.

11.2.14. After complete, take down the apparatus and rinse all parts with water. Use a damp paper towel to wipe down midi still and surrounding area.

Note: If Simultaneously Extracted Metals are to be determined, decant bubbler tube to 100 ml graduated cylinder, dilute to volume with water and pour into an acid cleaned plastic storage container for metals analysis.

11.3. Color development and analysis

11.3.1. If the sample is known or suspected to fall within the range of the curve, transfer the entire volume of tower A to a 50 ml centrifuge tube. Transfer 20 ml of the solution

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to a 30 ml centrifuge tube, add 2.5 ml of MDR and dilute to 25 ml with reagent water. For the six standards for the curve, add 5 ml MDR to 40 ml standard and dilute to 50 ml with reagent water in 50 ml centrifuge tubes.

If the samples are known or are suspected to be high in concentration, dilutions may be performed in 0.5N NaOH. Add 5 ml MDR to 40 ml diluted sample and dilute to 50 ml with reagent water.

11.3.2. If blue color change develops in the sample, the scrubber solution of tower B for that sample must also be analyzed. If no color change occurs for tower A, then tower B does not have to be analyzed.

11.3.3. For tower B add 5 ml MDR and dilute to 50 ml with reagent water.

11.3.4. After adding the MDR, allow a full thirty minutes for color development, but read the samples before 2 hours have elapsed.

11.3.5. Allow a thirty minute warm-up of the spectrophotometer. Zero the instrument with D.I. water at 670.0 nm.

11.3.6. Calibrate the instrument with the prepared curve starting with the low standard. A calibration with a correlation coefficient (r) of ≥ 0.995 must be obtained. Corrective action for a correlation coefficient < 0.995 is to discard existing curve, obtain new volumetrics and make a new curve. Verify concentration of stock sulfide standard.

11.3.7. Analyze samples in the following order:

Analysis	Sample
1	CCV-1
2	CCB-1
3	LCS-1
4	MB-1
5	Sample-1
6	Sample-1D
7	Sample-1S
8	Sample-1SD
9-11	Sample 2 - 4
12	CCV-2
13	CCB-2
14 -23	Sample 5 - 14
24	CCV-3
25	CCB-3

11.3.8. Analyze a continuing calibration verification standard (CCV) and continuing calibration blank (CCB) every 10 sample or QC analyses. The CCV is the fifth point

on the curve. The CCV must be ± 10% of the expected value. If the CCV fails, then the curve is not correct. The correlation coefficient may be ≥ 0.995, but if the slope is to high or too low, then the CCV will not pass. Notify supervisor, re-make the curve and re-calibrate.

11.3.9. The CCB must be < 0.05 ppm - The CCB is the first point of the curve.

11.3.10. Calculate AVS concentration as follows:

$$\frac{(Conc.\ from\ curve)(DF)\ x\ Final\ volume}{Initial\ wt\ x\ \%solids.} = mg/Kg\ AVS$$

11.3.11. Final volume when doing this procedure is 40 mL using the Midi apparatus and 100 mL using the Macro distillation procedure.

12. QA/QC REQUIREMENTS

12.1. Initial Precision and Recovery Validation

12.1.1. The accuracy and precision of the procedure must be validated before analyses of samples begin, or whenever significant changes to the procedures have been made. To do this four analyte free glass beads are spiked with the LCS spike solution, then prepared and analyzed. For each analyte the mean recovery of the true value should be 60-130%, and the RSD should be ≤ 20%.

12.2. Method Detection Limits and Method Reporting Limits

12.2.1. A method detection limit (MDL) study must be undertaken before analysis of samples can begin. To establish detection limits that are precise and accurate, the analyst must perform the following procedure. Spike seven blank matrix (water or soil) samples with MDL spiking solution at a level below the MRL. Follow the analysis procedures in Section 11 to analyze the samples.

12.2.2. Calculate the average concentration found in *mg/Kg AVS*, and the standard deviation of the concentrations (s) in *mg/Kg AVS.* Calculate the MDL for each analyte. Refer to the CAS *SOP Performing Method Detection Limit Studies and Establishing Limits of Detection and Quantification (ADM-MDL).* The MDL study must be verified annually.

12.2.3. Limits of Quantification (LOQ)

12.2.3.1. The laboratory establishes a LOQ for each analyte as the lowest reliable laboratory reporting concentration or in most cases the lowest point in the

calibration curve which is less than or equal to the desired regulatory action levels, based on the stated project requirements. Analysis of a standard or extract prepared at the lowest point calibration standard provides confirmation of the established sensitivity of the method. The LOQ recoveries should be within 50-150% of the true values to verify the data reporting limit. Refer to the CAS SOP *Performing Method Detection Limit Studies and Establishing Limits of Detection and Quantification (ADM-MDL)*

12.2.4. The Method Reporting Limits (MRLs) used at CAS are the routinely reported lower limits of quantitation which take into account day-to-day fluctuations in instrument sensitivity as well as other factors. These MRLs are the levels to which CAS routinely reports results in order to minimize false positive or false negative results. The MRL is normally two to ten times the method detection limit.

12.3. Ongoing QC Samples required are described in the CAS-Kelso Quality Assurance Manual and in the SOP for Sample Batches. Additional QC Samples may be required in project specific quality assurance plans (QAPP). General QC Samples are:

12.3.1. A method blank (MB) for every 20 samples analyzed.

12.3.2. One LCS for every 20 samples analyzed. Due to the time requirements for set-up and distillation, it is best to distill 2 LCS's for every 20 samples. Sulfides are very unstable and easily degraded and therefore it is not uncommon to have low sample recoveries.

12.3.3. Analyze one matrix spike and one matrix spike duplicate per 20 samples minimum.

12.3.4. Analyze one sample duplicate for every 20 samples minimum.

12.4. Acceptance Criteria

12.4.1. The method blank (MB) must be < MRL. If MB > MRL, reanalyze MB and all samples associated with the MB. If problems persist, attempt to determine source of contamination and perform reanalyses as necessary.

12.4.2. The MRL is calculated as follows:

$$\frac{0.05\ mg/L\ x\ Final\ volume(L)}{Initial\ wt.(kg)\ x\ \%solids}$$

12.4.3. The LCS must be 60-130% of the expected value. If the LCS recovery is outside of the criteria, sulfides were lost at some point during distillation, or sample was spiked at the improper level. Re-analyze LCS and all samples associated with that LCS. (Note - LCS's generally only fail high if the sample was spiked too high).

12.4.4. Matrix Spike recovery criteria is 30-160% recovery. If the recovery is outside the criteria, re-analyze spike. If two spikes were analyzed initially, then matrix interferences caused low spike recoveries. No need to reanalyze at that point.

12.4.5. The relative percent difference (RPD) for duplicates should be $\leq$ 20% RPD. If the RPD is > 20%, verify homogeneity of sample. Reanalyze the duplicate. Calculate RPD as follows:

$$RPD = \frac{High\ value\ -\ Low\ value}{Average}\ x\ 100$$

13. DATA REDUCTION, REVIEW, AND REPORTING

13.1. It is the analyst's responsibility to review analytical data to ensure that all quality control requirements have been met for each analytical run. Calculate samples results as described in section 11.6.12. Results for QC analyses are calculated and recorded as specified in section 12. Average, RPD, spike level and spike recovery are entered on benchsheet for corresponding samples. All data will be initialed, dated and attached to required data quality worksheet.

13.2. The appropriate benchsheets, located in Attachment A, should be in use at all times during AVS analysis.

13.3. The data packet for the sequence is submitted for review by supervisor or designee. The results are transferred to the appropriate report form located in the CAS network directory R:\WET\WIP. These forms are made from templates located in R:\WET\FORMS.

13.4. Data Review and Assessment

13.4.1. Following primary data interpretation and calculations, all data is reviewed by a secondary analyst. Following generation of the report, the report is also reviewed. Refer to the *SOP for Laboratory Data Review Process (ADM-DREV)* for details. The person responsible for final review of the data report and/or data package should assess the overall validity and quality of the results and provide any appropriate comments and information to the Project Chemist to inclusion in the report narrative.

13.5. Reporting

13.5.1. Soils, sediments, and other solid matrices are reported as µmol/g, either dry weight or as received, according to the project requirements.

AVS, µmol/g = (mg/Kg AVS in sample)/ [(32 g/mol S) (dry weight ratio) (sample weight g)]

13.5.2. The MRL is calculated as follows:

$$\frac{0.05\ mg/L\ x\ Final\ volume(L)}{Initial\ wt.(kg)\ x\ \%solids}$$

13.5.3. The MRL will vary depending on % solids content. For samples reported as received, the MRL = 0.05 mg/Kg.

13.5.4. For samples that did not require a dilution, 3 significant figures will be reported. For samples that required a dilution, report only 2 significant figures.

14. CONTINGENCIES FOR HANDLING OUT-OF-CONTROL OR UNACCEPTABLE DATA

14.1. Refer to the SOP for *Corrective Action (ADM-CA)* for procedures for corrective action. Personnel at all levels and positions in the laboratory are to be alert to identifying problems and nonconformities when errors, deficiencies, or out-of-control situations are detected.

14.2. Handling out-of-control or unacceptable data

14.2.1. On-the-spot corrective actions that are routinely made by analysts and result in acceptable analyses should be documented as normal operating procedures, and no specific documentation need be made other than notations in laboratory maintenance log books, run logs, for example.

14.2.2. Documentation of a nonconformity must be done using a Nonconformity and Corrective Action Report (NCAR) when:

- Corrective action is not taken or not possible
- Corrective action fails to correct an out-of-control problem on a laboratory QC or calibration analysis.
- Reanalysis corrects the nonconformity but is not a procedurally compliant analysis.

15. METHOD PERFORMANCE

15.1. Available method performance data is given in the reference method. In addition, this procedure was validated through single laboratory studies of accuracy and precision as specified in Section 12.1. The method detection limit(s) and method reporting limit(s) were established for this method as specified in Section 12.2.

16. POLLUTION PREVENTION

It is the laboratory's practice to minimize the amount of solvents and reagents used to perform this method wherever technically sound, feasibly possible, and within method requirements. Standards are prepared in volumes consistent with laboratory use in order to minimize the volume of expired standards to be disposed of. The threat to the environment from solvents and/or reagents used in this method may be minimized when recycled or disposed of properly.

17. WASTE MANAGEMENT

17.1. The laboratory will comply with all Federal, State, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions as specified in the CAS EH&S Manual.

17.2. This method uses acid. Waste acid is hazardous to the sewer system and to the environment. All acid waste must be neutralized to a pH of 5-9 prior to disposal down the drain. The neutralization step is considered hazardous waste treatment and must be documented on the treatment by generator record. See the CAS EH&S Manual for details.

17.3. This method uses a base. Waste base is hazardous to the sewer system and to the environment. All waste must be neutralized to a pH of 5-9 prior to disposal down the drain. The neutralization step is considered hazardous waste treatment and must be documented on the treatment by generator record. See the CAS EH&S Manual for details

18. TRAINING OUTLINE

18.1. Training outline – Training Plan

18.1.1. Review literature (see references section). Read and understand the SOP. Also review the applicable MSDS for all reagents and standards used. Following these

reviews, observe the procedure as performed by an experienced analyst at least three times.

18.1.2. The next training step is to assist in the procedure under the guidance of an experienced analyst for a period of two months. During this period, the analyst is expected to transition from a role of assisting, to performing the procedure with minimal oversight from an experienced analyst.

18.1.3. Perform an Initial Demonstration of Capability (IDOC) as described above. Summaries of the IDOC are reviewed and signed by the departments Technical Services Manager. Copies of the IDOC and Training Plan are forwarded to QA for record keeping. For applicable tests, IPR studies are performed in order to be equivalent to NELAC's Initial Demonstration of Capability.

18.1.4. Training is documented following the SOP *for Documentation of Training*.

18.1.4.1. When the analyst training is documented by the supervisor on internal training documentation forms, the supervisor is acknowledging that the analyst has read and understands this SOP and that adequate training has been given to the analyst to competently perform the analysis independently.

19. METHOD MODIFICATIONS

19.1. No method modification noted for Revision 6 of this SOP.

20. REFERENCES

20.1. *Draft Analytical Method for Determination of Acid Volatile Sulfide in Sediment,* June, 1997 ;
EPA Method 1629.

20.2. *Measurement of Bacterial Sulfate Reduction in Sediments: Evaluation of a Single-step Chromium Reduction Method*, Henrik Fossing and Bo Barker Jorgensen, 1989, *Biochemistry*.

21. CHANGES SINCE THE LAST REVISION

21.1. Sec 1.4 - Removed the third sentence that was in previous revision
21.2. Sec 3.1 – 3.7 is new
21.3. Sec 7.1.1.1 – corrected and updated how to set up Midi still
21.4. Sec 7.3 is new
21.5. Sec 8.1 is new
21.6. Sec 8.3.2.1 changed 10 mL to 11 mL and added *or beakers*

21.7. Sec 8.4.4 – corrected equation
21.8. Sec 8.9.2.2 – changed 10.8 to 10.87
21.9. Sec 11.2.1 replaced 6 N HCL with 10% HNO3
21.10. Sec 11.2.2 – replaced the word system with apparatus
21.11. Sec 11.2.6 is new
21.12. Sec 11.2.10.2 – replace sand with glass beads
21.13. Sec 11.2.14 – NOTE updated
21.14. Sec 11.3.3-removed transfer to 30 mL centrifuge tube, changed 2.5 mL to 5.0 and 25 to 50 mL
21.15. Sec 11.3.11 – re-wrote for clarity
21.16. Sec 12.1 – 12.2 is new
21.17. Sec 14 - updated
21.18. Sec 19 and 21 are new

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APPENDIX A

Procedure for Chromium Reducible Sulfur

1. After removing A-tower scrubbers and B-towers from the AVS distillation, replace them with new A and B towers described in Sections 11.2.3 and 11.2.8.

2. Using a syringe and needle, add 5mL of ethanol to each sample tube through the septum.

3. Purge system for 15 minutes with nitrogen gas.

4. Add 16mL of Cr^{+2} solution to each sample tube through the septum using syringes and needles prepared in the nitrogen glove box.

   Note: The aliquots (in the syringes) of the Cr^{+2} solution must be prepared in the nitrogen glove box to prevent the solution from reducing back to Cr^{+3}.

5. Add 8mL of concentrated HCl using syringes and needles.

6. Distill at room temperature for 15 minutes, then increase the temperature of midi to 90°C. Distill for an additional 45 minutes.

7. The concentration of the A and B towers are determined as for AVS using the procedure in Section 11.3.

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ATTACHMENT A

Benchsheet

RESEARCH ENVIRONMENTAL & INDUSTRIAL CONSULTANTS, INC.
Post Office Box 286 • Beaver, WV 25813 • 800.999.0105
304.255.2500 • 304.255.2572(fax)
website: www.reiclabs.com
Improving the environment, one client at a time...

Standard Operating Procedures
Benthic Macroinvertebrate Sorting & Taxonomic Identification

Research, Environmental, and Industrial Consultants, Inc., (REIC) has developed standard operating procedures (SOP) to process benthic macroinvertebrate samples in the laboratory for the purpose of measuring the taxonomic composition and abundance of benthic macroinvertebrates found in freshwaters. These SOP's are based on several standard laboratory practices, including those used by the U.S. Environmental Protection Agency (USEPA), and the West Virginia Department of Environmental Protection (WV- DEP) Watershed Branch (2009). In an effort to promote consistent bioassessments of water quality, the USEPA has produced Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers (Barbour et al, 1999) for use in collecting and processing benthic macroinvertebrate samples from streams and rivers. The REIC "Standard RBP Methodology" and "Modified RBP Methodology" benthic macroinvertebrate laboratory SOP's borrow heavily from the USEPA protocols, but incorporate modifications based on REIC's experience. This SOP describes the 200-count sub-sampling typically used by the benthic macroinvertebrate laboratory of REIC to process benthic macroinvertebrate samples.

Quality Assurance and Quality Control

All laboratory personnel will receive basic instruction and evaluation in the sample processing procedure by experienced laboratory staff. Quality Control (QC) procedures are used to ensure that the data consists of <10% total error for the extraction of benthic macroinvertebrates from samples and <10% total error for the identification and the enumeration of the extracted organisms.

QC of sample set-up, extraction, sorting, and cleanup

The Quality Control procedure for monitoring taxonomic sample set-up, extraction, and sorting of benthic macroinvertebrates uses a re-sort method to identify unacceptable (>10%) levels of error in the data, and implements corrective actions that decrease the data error to acceptable levels (<10%). Each project received in the laboratory will receive QC on at least 10% of its samples.

An experienced QC officer will check all sorted grids from every 10^{th} sample processed by a sorter to ensure that each meets the >90% sorting efficiency. Qualification will only occur when a sorter achieves >90% sorting efficiency for five samples consecutively.

The QC officer will calculate percent sorting efficiency (PSE) for each sample as follows: PSE = A / (A +B) x 100 where A = the number of organisms found by the primary sorter and B = the number of organisms missed by the primary sorter and found during the QC check.

A PSE > or = 90% is considered passing.

If the sorting efficiency for each of these five consecutive samples is >90% for a particular individual, this individual is considered "experienced."

In the event that an individual fails to achieve >90% sorting efficiency, they will be required to sort an additional five samples and their sorting efficiency will continue to be monitored until they become "experienced."

Even after individuals qualify, 10% of all of their sorted samples will be checked for sorting efficiency.

If an "experienced" individual fails to maintain a >90% sorting efficiency as determined by QC checks, QC checks will be performed on every grid of five consecutive samples until a > 90% sorting efficiency is achieved on all five.

QC of taxonomic identification and enumeration

Every scientist will achieve and maintain at least 90% accuracy rating in macroinvertebrate identification.

Each scientist will have each sample site bottle that he/she identified to genus level reviewed in its entirety by the laboratory administrator or a senior taxonomist until he/she achieves 90% accuracy rating to the family and genus levels. At this point the taxonomist will be considered "experienced."

Each "experienced" scientist in the lab will perform a 10% sample exchange on all samples and Percent Difference in Enumeration and Percent Taxonomic Disagreement will be calculated for each sample.

Percent Difference in Enumeration (PDE) will be calculated for each sample as follows: (n1 – n2) / (n1 + n2) x 100, where n1 is the number of specimens counted in a sample by the first scientist and n2 is the second scientist. The purpose is to find the samples where counts differ and determine the reason for the miscounts. The PDE for each sample checked will be entered into a database and kept indefinitely. The goal of REIC is for each scientist to achieve 90% agreement in enumeration for each sample identified.

A PDE < or = 10% is considered passing.

Percent Taxonomic Disagreement (PTD) will be calculated for each sample as follows: 1 – (number of agreements / N) x 100, where N is the total number of specimens in the larger of the two counts. Agreements are determinant upon the targeted level of identification. For example, if family is the target, and one taxonomist provides a name for a specimen at the species level and the other leaves the name at the family level, it would constitute an agreement. However, if genus is the target, and one taxonomist identifies at the genus level, and the other identifies it at the family level, it would not be scored as an agreement. The PTD for each sample checked will be entered into a database and kept indefinitely. The goal of REIC is for each scientist to agree 90% of the time in family and genus level identification.

A PTD < or = 10% is considered passing for Family Level taxonomy.
A PTD < or = 15% is considered passing for Genus Level taxonomy.

If a scientist is unable to consistently perform at the designated standards, he/she will be instructed on an individual basis. If the scientist fails to improve to the designated standards, a written disciplinary report will go into his/her employee file. If after individual instruction, the scientist does not improve, he/she will no longer be permitted to identify macroinvertebrates in the lab until he/she demonstrates an ability to adhere to the set standards

A reference collection will be established and stored in designated cabinets in the benthic macroinvertebrate laboratory. The collection will consist of representative specimens of each species to the lowest identifiable taxon (i.e., genus, family). In addition, species new to the laboratory's reference collection will be sent to recognized experts for taxonomic verification. The verified specimens will then be added to the collection. All specimens in the reference collection will be preserved in 70% ethyl alcohol in glass vials with labels made of waterproof paper printed with a laser printer. Reference specimens will be organized within major taxonomic groups. The laboratory administrator will maintain the collection log including the organism name, the location of the reference specimen, the status of the specimen if it has been loaned to outside experts, and information about confirmation by outside experts.

At least one scientist in the laboratory will obtain North American Benthological Society (NABS) certification in Ephemeroptera, Plecoptera, Trichoptera. The laboratory administrator will provide the tools necessary for the scientists to achieve the required accuracy ratings.

Standard RBP Methodology **sample rinsing and set-up, sorting, and cleanup**

Rinsing and set-up:

1. Obtain a sample from the appropriate box on the designated shelf.
2. Record the sample label information, date of sample rinse and sorting, and the initials of the person rinsing and sorting the sample on the laboratory log sheet for the designated project and on the Benthic Macroinvertebrate Sample Sort sheet.
3. Pour and spread the sample evenly onto a U.S. standard soil sieve #35 (500µm) held above a waste bucket in the sink. Use large forceps if necessary to empty the sample container and spread the sample over the sieve. Drain the alcohol solution into the bucket and gently rinse the sample with water, draining the rinse water into the bucket until the water runs clear. Rinse the sample bottle through the sieve into the bucket to collect any residue remaining in the sample jar.
4. Remove the rinsed sample from the sieve to the gridded sorting tray, back rinse the sieve to collect all sample residues into the tray and add enough water to the tray to cover the sample.
5. If the sample contains a low amount (one jar) of inorganic substrate such as sand or gravel, proceed in the following manner. Transfer the sample material to the gridded pan and spread the contents evenly across the pan. If the amount of leaf litter or other detritus material exceeds that which fills the gridded pan, then divide the sample among two or more gridded pans.
6. If the sample contains a large amount (two or more jars) of inorganic substrate such as sand or gravel, carefully empty each sample jar into its own gridded pan.
7. Backwash the sieve to prevent cross-contamination of samples.

8. After the sediment from rinsing the sample has settled to the bottom of the waste bucket in the sink, dump the water from the top of the bucket through the sieve into the drain, reserving the sediment at the bottom of the bucket. Add water to the bucket and dump the bucket and sediment behind the building in the gravel area designated for samples.
9. Transport rinsed samples to the benthic macroinvertebrate laboratory to begin the sub-sampling procedure. Quantitative samples are sub-sampled to a randomized fixed count of 200 +/-20% benthic macroinvertebrate specimens per sample using standard EPA laboratory sorting methods or sorted whole if the target number of 200 is not reached. All sub-sampling is done using the 100-grid sorting trays. If a sample is spread between two or more sorting pans, repeat each of the following steps on each pan, combining the organisms from each gridded pan into one white sorting pan.

Sorting:

1. Remove large objects (sticks, stones, empty clam shells) and carefully inspect them under the 3X magnifying ring light for organisms. Return organisms, if found, to the sample in the gridded pan and place the large objects into a residue bucket. Gently spread the sample material over the bottom of the gridded pan as evenly as possible. Move the sample material into the corners of the gridded screen using forceps. Gently vibrate or shake the pan to help spread the sample.
2. Use a random number generator (numbered bottle caps) to select four grids (subsample) from the gridded tray. The goal is to randomly select at least four of the grids from the 100 grids on the gridded screen in an effort to ensure that the subsample material is representative of the overall sample. Record the total number of randomly chosen grids on the Benthic Macroinvertebrate Sample Sort Sheet.
3. Place the stainless steel dividing frame over the sample at the approximate locations of the grids selected for processing based on the numbers marked on the gridded tray.
4. Remove or extract the sample material within the dividing frame (Step 3) from each of the four grids using a white plastic teaspoon, mesh scoop, and/or forceps. Place the extracted sample material into a white plastic pan and add water to cover the sample material. Rinse the extraction tools and the inside surface of the dividing frame over the pan and inspect these tools and the grid for any remaining organisms using the 3X magnifying ring light.

   Use the following rules when dealing with organisms that lie on the line between two grids:

   - An organism belongs to the grid containing its head.
   - If it is not possible to determine the location of the head (i.e., for worms), the organism is considered to be in the grid containing most of its body
   - If the head of an organism lies on the line between two grids, all organisms on the top border of a grid and those on the right border of a grid belong in that grid, and are picked with that grid.

5. Set the gridded tray aside until the macroinvertebrates have been sorted and counted from the previously extracted grid material.
6. If the number of organisms within the first four grids appears to be lower than the sample target count (200 +/-20%), proceed in the following manner:

- If after sorting and counting all the organisms from the first four grids the organism count is greater than 140, put aside the organisms for that count and randomly choose a new grid following step 2.
- If four complete grids have yielded fewer than 30 organisms, group the next four randomly chosen grids and sort and count the target organisms.

If the organism count from the first eight grids is lower than 60, extract the entire remaining sample from the gridded pan and sort and count the benthic macroinvertebrates from it. Write in the "Number of Grids Picked" section of the Benthic Macroinvertebrate Sample Sort sheet that all grids were combined for sorting. If the organism count from the first six grids is higher than 120, resume random selection of individual grids until the target count (200 +/-20%) is reached. If it is uncertain whether a sample should be processed whole, it is at the discretion of the laboratory supervisor to determine whether a sample can be processed whole or by individual grids.

7. If the number of organisms in any four grids appears to exceed the target count (200 +20%), spread the sorted organisms in a second gridded pan and randomly choose individual grids, picking the organisms from each grid until the expected target count (200 +20%) is reached. Once a grid is chosen, the entire grid must be picked. Document in the "General Comments" section of the Benthic Macroinvertebrate Sample Sort sheet that this procedure was used.
8. Obtain a 60ml bottle from the bottle storage area and label it with the appropriate sample label from the project clipboard in the laboratory. Add approximately 50ml of 70% Ethyl Alcohol.
9. Slowly search the entire pan in a systematic pattern to locate all identifiable benthic macroinvertebrates. First search the base of the pan and then search focusing on the surface of the water, looking for surface floating organisms. Remove organisms and place in the appropriately labeled 60ml bottle keeping a tally of the number of organisms placed in the bottle. Record the total number of organisms removed from the sample in the appropriate space on the Benthic Macroinvertebrate Sample Sort Sheet.
10. Do not remove or count: empty snail or bivalve shells; empty caddisfly cases; fragments such as legs, antennae, gills, wings, or headless bodies; round worms (Nematoda); microcrustacea (copepods, ostracods, branchiopods); eggs; or winged adult aquatic insects (except Coleoptera). Search inside empty snail and bivalve shells and caddisfly cases for the presence of smaller target organisms. Also, search inside aquatic plant stems and leaves for small invertebrates such as Diptera larvae and pupae that mine such tissues. Insects thought to be terrestrial should be verified as such by a taxonomist at sorting time or placed in the labeled bottle for later verification but should not be counted. For segmented worms (Oligochaeta) remove and count only whole bodies and fragments that include a rounded end that could be a head or tail end. Count Oligochaeta end fragments as1/2 counts (two ends equals one count). Count a whole worm as one count. If unsure as to whether any specimen should be counted, place the organism in the labeled bottle without counting it (the final identity and count will be made by a taxonomist).
11. Record the date each sample was sorted in the appropriate space on the Benthic Macroinvertebrate Sample Sort sheet. Keep a record of the amount of time spent sorting each sample and record it in the appropriate space on the Benthic Macroinvertebrate Sample Sort sheet. Record the total number of grids chosen in the appropriate space on the Benthic Macroinvertebrate Sample Sort Sheet.
12. Prior to discarding the remaining un-sorted sample, briefly look through the sample and record the presence of any large or obviously abundant organisms in the appropriate space on the Benthic Macroinvertebrate Sample Sort Sheet.

13. Place the completed Benthic Macroinvertebrate Sample Sort sheet in the project specific binder with the Benthic Macroinvertebrate Sample Log-in form.
14. Record the date sorted, initials of the person sorting, and the number of hours spent sorting the sample on the laboratory log sheet on the clipboard in the lab.

Sample cleanup:

- Clean the gridded pan using dish soap or all-purpose cleaner and a rag if needed and then backwash the screen.
- Thoroughly wash and rinse all sorting equipment used including pipefittings, scoops, bowls, etc.
- Thoroughly wipe the laboratory table using all purpose cleaner and paper towels.

Sample Storage

All identified samples (macroinvertebrates only) are kept in storage at REIC for at least two years. All picked samples (detrital material) are generally kept only for 6 months to 1 year, and then are dumped and disposed of appropriately.

Quality Control Sheet

Percent Sorting Efficiency (PSE)

The Percent Sorting Efficiency (PSE) (AKA Bias) was calculated by the following formula:

$$\frac{\text{\# Organisms Originally Sorted}}{\text{\# Organisms Recovered By Checker + \# Organisms Originally Sorted}}$$

PSE =

Percent Sorting Efficiency (PSE) A PSE >= 90% is considered passing.

Percent Difference in Enumeration (PDE)

The Percent Difference in Enumeration (PDE) is calculated by the following formula:

$$PDE = \left(\frac{|\ n1\ -\ n2\ |}{n1\ +\ n2} \right) \times 100$$

PDE =

Percent Difference in Enumeration (PDE) Where: n_1 = # of organisms counted by taxonomist 1 n_2 = # of organisms counted by taxonomist 2. A PDE <=10% is considered passing.

Percent Taxonomic Difference (PTD)

$$PTD = \left[1 - \left(\frac{comp_{pos}}{N} \right) \right] \times 100$$

PTD =

Where: N= Highest count of organisms from taxonomist 1or 2. $comp_{pos}$ = Total # of taxonomic agreements from the Taxonomic Comparison Form.

A PTD <=10% is considered passing for Family Level taxonomy.

A PTD <=15% is considered passing for Genus Level taxonomy.

Taxonomic References
for Benthic Macroinvertebrate Identification

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Voshell, J.R., Jr. 2005. A Guide to Common Freshwater Invertebrates of North America. The McDonald and Woodward Publishing Company, Blacksburg, Virginia.

Wiggins, Glenn B. 1977. Larvae of North American Caddisfly Genera (Trichoptera). University of Toronto Press, Toronto.

Ohio EPA Supplemental Keys to the Larval Chironomidae (Diptera) of Ohio and Ohio Chironomidae Checklist. June 2007, Michael J. Bolton.

Epler, J.H. 2001. Identification Manual for the larval Chironomidae (Diptera) of North and South Carolina. A guide to the taxonomy of the midges of the southeastern United States, including Florida.

Merritt R.W, Cummins K.W, and Berg M.B (eds). 2007. Aquatic insects of North America, 4th Edition. Kendall/Hunt, Dubuque.

Wiederholm, T. (Ed.): Chironomidae of the Holarctic region. Keys and diagnoses. Vol. 1. Larvae. 1983.

Minter J., Jr. Westfall, Michael L. May. Damselflies of North America

Aquatic Entomology: The Fishermen's Guide and Ecologists' Illustrated Guide to Insects and Their Relatives, W. Patrick McCafferty.

Pennak's Freshwater Invertebrates of the United States: Porifera to Crustacea, 4th Edition.

CHAIN OF CUSTODY
BIOLOGICAL SAMPLES

Client Name: _____ Project Name or ID #: _____

Date of Collection: _____ Collector(s): _____

Collection Site(s) [Use Stream Names and Site Names]:

Stream Name(s): _____

County: _____

Type of Samples Collected (Circle One or More):
Benthic Macroinvertebrate Fish Plants Other: _____

Method of Collection (Check all that apply):
Benthics: Rectangular Kick (4 reps) Surbers PIBS Kick-Seine Other: _____
Fish: Backpack Electrofishing Boat Electrofishing Seining Other: _____

Samples Preserved In (Circle One): Formalyn Alcohol Not Preserved Other: _____

Samples Included with this Chain of Custody: [Each sample to be listed]

Samples Delivered By (Method & Persons): _____

Samples Relinquished By: _____ Date/Time: _____

Samples Received By: _____ Date/Time: _____

C Supporting Documents

Benthic Data Collection and Biological Monitoring Datasheets

18.1 FIELDSHEET FOR THE COLLECTION OF BIOLOGICAL MONITORING DATA

New York State Department of Environmental Conservation

FIELD DATA SHEET **4-letter identifier** _____

STREAM / STATION _____ **CITY/TOWN/VILLAGE** _____
ROUTE NO. _____
DATE _____
TIME : ARRIVAL _____ **UNIQUE FEATURES** _____
DEPARTURE _____
COLLECTORS _____ **SITE TYPE:** **RIBS SCREENING** _____
LATITUDE\ LONGITUDE _____ **RIBS INTENSIVE** _____
MULTI-SITE SURVEY _____

PHYSICAL AND CHEMICAL PARAMETERS

DEPTH (meters) _____ TEMPERATURE (ºC) _____

WIDTH (meters) _____ SPEC. CONDUCT. (µmhos) _____

CURRENT (cm/sec) _____ pH _____

CANOPY (%) 0 10 25 50 75 90 100 D.O. (mg/l; ppm) _____ / sat. _____ %

EMBEDDEDNESS (%) _____ SALINITY _____

SECCHI DISK _____

SUBSTRATE: (%) Rock _____ Rubble _____ Gravel _____ Sand _____ Silt _____

AQUATIC VEGETATION: Algae (suspended) _____ Algae (filamentous) _____

Diatoms (on rocks) (%) _____ Thickness _____ Macrophytes (%) _____

TYPE OF SAMPLE **OCCURRENCE OF MACROINVERTEBRATES**

Multiplate	_____	Ephemeroptera	_____	Chironomidae	_____
Kick, sample retained	_____	Plecoptera	_____	Simuliidae	_____
Kick, sample not retained	_____	Trichoptera	_____	Decapoda	_____
Ponar	_____	Coleoptera	_____	Gammaridae	_____
Organisms for toxics	_____	Megaloptera	_____	Mollusca	_____
Photograph	_____	Odonata	_____	Oligochaeta	_____
Microtox sample	_____	Other	_____	_____	
Other	_____				

FAUNAL CONDITION: very good _____ good _____ poor _____ very poor _____
Habitat: adequate _____ impoundment _____ headwater _____ sand _____ gravel _____
bedrock _____ low flow _____ other _____
Landuse: Residential _____ Agriculture _____ Commmerical _____ Industrial _____
Forest _____ Recreational _____ Wetland _____

NOTES, OBSERVATIONS	RIBS SCREENING SITE CRITERIA
	1. Mayflies (3 or more taxa) _____
	2. Stoneflies (present) _____
	3. Caddisflies (less abund. than mayflies) _____
	4. Beetles (present) _____
	5. Worms (sparse or absent) _____

BENTHIC MACROINVERTEBRATE SAMPLE LOG-IN SHEET

Date Collected	Collected By	Number of Containers	Preservation	Station #	Stream Name and Location	Date Received by Lab	Lot Number	Date of Completion		
								sorting	mounting	identification

Serial Code Example: B0754001(1)
B = Benthos (F = Fish; P = Periphyton) ■ 0754 = project number ■ 001 = sample number ■ (1) = lot number (e.g., winter 1996 =1; summer 1996 = 2)

FISH SAMPLING FIELD DATA SHEET (FRONT)

STREAM NAME	LOCATION
STATION #________ RIVERMILE________	STREAM CLASS
LAT ____________ LONG ____________	RIVER BASIN
STORET #	AGENCY
GEAR	INVESTIGATORS
FORM COMPLETED BY	DATE ______ TIME ______ AM PM — REASON FOR SURVEY

SAMPLE COLLECTION	**How were the fish captured?** ❑ back pack ❑ tote barge ❑ other ____________ **Block nets used?** ❑ YES ❑ NO **Sampling Duration** Start time ________ End time ________ Duration ________ **Stream width (in meters)** Max________ Mean________
HABITAT TYPES	**Indicate the percentage of each habitat type present** ❑ Riffles____% ❑ Pools____% ❑ Runs____% ❑ Snags____% ❑ Submerged Macrophytes____% ❑ Other ( )____%
GENERAL COMMENTS	

SPECIES	TOTAL (COUNT)	OPTIONAL: LENGTH (mm)/WEIGHT (g) (25 SPECIMEN MAX SUBSAMPLE)					ANOMALIES*							
							D	E	F	L	M	S	T	Z

FISH SAMPLING FIELD DATA SHEET (BACK)

SPECIES	TOTAL (COUNT)	OPTIONAL: LENGTH (mm)/WEIGHT (g) (25 SPECIMEN MAX SUBSAMPLE)					ANOMALIES*							
							D	E	F	L	M	S	T	Z

* **ANOMALY CODES:** D = deformities; E = eroded fins; F = fungus; L = lesions; M = multiple DELT anomalies; S = emaciated; Z = other

Habitat Condition Observations Datasheet

18.4 FIELDSHEET FOR RAPID ASSESSMENT OF HABITAT CONDITION IN LOW GRADIENT STREAMS

New York State Department of Environmental Conservation
Field Sheet for Rapid Assessment of Habitat Condition (Low Gradient)

Stream Name:_____
4-letter Identifier/Station Number:_____
Collectors:_____
Biological Sample: Y N
Site Type: Screening Intensive Multi-Site

Parameters to be evaluated in sampling reach	Habitat Parameter	Condition Category			
		Optimal	**Suboptimal**	**Marginal**	**Poor**
	1. Epifaunal Substrate/ Available Cover	Greater than 50% of substrate favorable for epifaunal colonization and fish cover, mix of snags, submerged logs, undercut banks, cobble or other stable habitat and at stage to allow full colonization potential (i.e. logs/snags that are not new fall and not transient)	30 – 50% mix of stable habitat; well-suited for full colonization potential, adequate habitat for maintenance of populations; presence of additional substrate in the form of new fall, but not yet prepared for colonization (may rate at high end of scale).	10 – 30 % mix of stable habitat; habitat availability less than desirable, substrate frequently disturbed or removed.	Less than 10 % stable habitat; lack of habitat is obvious; substrate unstable or lacking.
	SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
	2. Pool Substrate Characterization	Mixture of substrate materials, with gravel and firm sand prevalent; root mats and submerged vegetation common.	Mixture of soft sand, mud, or clay; mud may be dominant; some root mats and submerged vegetation present.	All mud or clay or sand bottom; little or no root mat; no submerged vegetation.	Hard-pan clay or bedrock; no root mat or vegetation.
	SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
	3. Pool Variability	Even mix of large-shallow, large-deep, small-shallow, small-deep pools present.	Majority of pools large-deep; very few shallow.	Shallow pools much more prevalent than deep pools.	Majority of pools small-shallow or pools absent.
	SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
	4. Sediment Deposition	Little or no enlargement of islands or point bars and less than 20% of the bottom affected by sediment deposition.	Some new increase in bar formation, mostly from gravel, sand or fine sediment; 20-50% of the bottom affected; slight deposition in pools.	Moderated deposition of new gravel, sand or fine sediment on old and new bars; 50-80% of the bottom affected; sediment deposits at obstructions, constructions, and bends; moderate deposition of pools prevalent.	Heavy deposits of fine material, increased bar development; more than 80% of the bottom changing frequently; pools almost absent due to substantial sediment deposition.
	SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
	5. Channel Flow Status	Water reaches base of both lower banks, and minimal amount of channel substrate is exposed.	Water fills >75% of the available channel; or <25% of channel substrate is exposed.	Water fills 25-75% of the available channel, and/or riffle substrates are mostly exposed.	Very little water in channel and mostly present as standing pools.
	SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0

Parameters to be evaluated in sampling reach

Habitat Parameter	Condition Category			
	Optimal	**Suboptimal**	**Marginal**	**Poor**
6. Channel Alteration	Channelization or dredging absent or minimal; stream with normal pattern.	Some channelization present, usually in areas of bridge abutments; evidence of past channelization, i.e., dredging, (greater than past 20 yr) may be present, but recent channelization is not present.	Channelization may be extensive; embankments or shoring structures present on both banks and 40 to 80% of stream reach channelized and disrupted.	Banks shored with gabion or cement; over 80% of the stream reach channelized and disrupted. Instream habitat greatly altered or removed entirely.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
7. Channel Sinuosity	The bends in the stream increase the stream length 3 to 4 times longer than if it was in a straight line. (Note – channel braiding is considered normal in coastal plains and other low-lying areas. This parameter is not easily rated in these areas.	The bends in the stream increase the stream length 2 to 3 times longer than if it was in a straight line.	The bends in the stream increase the stream length 1 to 2 times longer than if it was in a straight line.	Channel straight; waterway has been channelized for a long time.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
8. Bank Stability (score each bank)	Banks stable; evidence of erosion or bank failure absent or minimal; little potential for future problems. <5% of bank affected.	Moderately stable, infrequent, small areas of erosion; mostly healed over. 5-30% of bank in reach has areas of erosion.	Moderately unstable; 30-60% of bank in reach has areas of erosion potential during floods.	Unstable, many eroded areas; "raw" areas frequent along straight sections and bends; obvious bank sloughing; 60-100% of bank has erosional scars.
SCORE___(LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE___(RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0
9.Vegetative Protection (score each bank)	More than 90% of the streambank surfaces and immediate riparian zone covered by native vegetation, including trees, understory shrubs, or nonwoody macrophytes; vegetative disruption through grazing or mowing minimal or not evident; almost all plants allowed to grow naturally.	70-90% of the streambank surfaces covered by native vegetation, but one class of plants is not well-represented; disruption evident but not affecting full plant growth potential to any great extent; more than one-half of the potential plant stubble height remaining.	50-70% of the streambank surfaces covered by vegetation; disruption obvious; patches of bare soil or closely cropped vegetation common; less than one-half of the potential plant stubble height remaining.	Less than 50% of the streambank surfaces covered by vegetation; disruption of streambank vegetation very high vegetation has been removed to 5 centimeters or less in average stubble height.
SCORE___(LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE___(RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0
10. Riparian Vegetative Zone Width (score each bank riparian zone)	Width of riparian zone >18 meters; human activities (i.e., parking lots, roadbeds, clear-cuts, lawns, or crops) have not impacted zone.	Width of riparian zone 12-18 meters; human activities have impacted zone only minimally.	Width of riparian zone 6-12 meters; human activities have impacted zone a great deal.	Width of riparian zone <6 meters; little or no riparian vegetation due to human activities.
SCORE___(LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE___(RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0

Note: determine left or right side by facing downstream.

Fish Sampling and Data Collection Datasheet

ecology and environment, inc.

Fisheries Sampling and Field Data Collection Form

Project Information

Project Name: _____ **Date: Time:** _____

Project Number: _____ **Weather** _____

Plant Location: _____ **Conditions: Air** _____

Field Team Leader: _____ **Temperature (°F):** _____

Field Team Members: _____ **Precipitation:** _____

H&S Daily Meeting: (topics covered)

Comments (observations, visitors - names, arrival/departure times): _____

Water Quality/Physical Data

Source Water Body: _____ **River/Lake Stage:** _____ **Other:** _____

Temperature: _____ **DO:** _____ **Temperature:** _____ **pH:** _____ **Conductivity:** _____

Fisheries Data

Sampling Method: _____ **Sample Location:** _____

Sample ID: _____ **Effort (electofishing - mins; gill/hoop nets - hours; etc.):** _____

Sample ID	Length (mm)	Weight (g or lbs)	Condition (Live, Dead, Moribund)	Abnor-malities	Batch Length Category (mm)	Batch Count	Batch Weight (g or lbs)	Voucher	Comments/Description of Sampling Conditions

Signature: _____ **Date:** _____ **Page** ____ **of** ____

ecology and environment, inc.

Fisheries Sampling and Field Data Collection Form

Project Information

Project Name: _____ **Date:** _____

Project Number: _____ **Field Team Leader:** _____

Plant Location: _____ **Field Team Members:** _____

Fisheries Data

Sampling Method: _____ **Sample Location:** _____

Sample ID: _____ **Other:** _____

Sample ID	Length (mm)	Weight (g or lbs)	Condition (Live, Dead, Moribund)	Abnor-malities	Batch Length Category (mm)	Batch Count	Batch Weight (g or lbs)	Voucher	Comments/Description of Sampling Conditions

Signature: _____ **Date:** _____ **Page ____ of ____**