Project-Specific Quality Assurance Project Plan for the Eighteen Mile Creek Site – OU2 Remedial Investigation/Feasibility Study Lockport, New York

Contract No. EP-W-10-007

April 2015

Prepared for:

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
Region 2
290 Broadway
New York, New York 10007-1866

Prepared by:

ECOLOGY AND ENVIRONMENT, INC.
368 Pleasant View Drive
Lancaster, New York 14086

Los Alamos Technical Associates, Inc.

Table of Contents

Section **Page**

Table of Contents (cont.)

Section **Page**

List of Tables

List of Tables (cont.)

List of Figures

Figure **Page**

QAPP Worksheet #11a – Project/Data Quality Objectives

Fish Tissue

Elevated PCBs, metals and PAHs were found in the sediment samples collected from the Creek Corridor as described the Revised Work Plan (Revision 01). Fish tissue samples are required to assess exposure pathways for the HHRA and BERA.

Fish DQOs include the following:

- Determine the concentrations of COPCs in the tissue of sport fish likely caught and eaten by the local population to assess the bioaccumulation pathway.
- Determine concentrations of COPCs in forage fish to access bioaccumulation pathway for piscivorous wildlife.
- Follow NYSDEC guidelines for fish sampling (i.e.., skin on fillets when collecting sport fish for contaminant monitoring [New York State Energy Research and Development Authority (NYSERDA) 2008]). See Appendix A.
- Follow any restrictions specified in the NYSDEC permit for fish collection.
- There are no risk screening values for fish but % lipids analysis is required in order to assess bioaccumulation factors. Quantitation limit goals listed for soil in Worksheet 15 are the same as for fish tissue.

Fish Sampling Boundaries of the Study are as follows:

The area for fish sampling is bounded by the Creek Corridor (see Figure 10 and 10a). Fish collected anywhere within the creek waters are considered to part of the same location. The fish sampling will focus on deeper areas of the creek that are likely to support a population of larger sport fish.

Sediments and Surface Waters

PCBs, metals, pesticides and PAHs were found in the sediment samples collected from the Creek Corridor at concentrations that indicate the potential for both acute and chronic toxicity impacts based on comparison with screening criteria. Toxicity testing is needed to determine if actual impacts exist. Acid volatile sulfides/simultaneously extracted metals (AVS/SEM) and organic carbon to in sediment will be measured to help assess the bioavailability of divalent metals including cadmium, copper, lead, nickel, zinc, and monovalent silver, and mercury.

Sufficient sample data are available for the creek channel to assess sediment contaminant exposure for the majority of analytical parameters, but there is not sufficient data for all parameters. Background sediment samples also are needed for statistical comparison to existing OU2 sediment data. Except for a few surface water samples collected for PCB analysis, there are no surface water contaminant data for OU2.

Sediment and Surface Water DQOs include the following:

- The samples collection should cover a range of contaminant concentrations (low, medium, high) so that both toxic and non-toxic samples are collected.
- Toxicity testing should address both potentially acute and chronic impacts.

- Quantitation limit goals listed for soil in Worksheet 15 are considered acceptable for sediment. However, sediment data also will be evaluated using criteria listed in "Screening and Assessment of Contaminated Sediment," issued by NYSDEC June 24, 2014. These sediment criteria are higher than the soil criteria.
- Quantitation limit goals listed for groundwater in Worksheet 15 are considered acceptable for surface water concentrations except for PCBs, for which lower detection limits are required.

Sediment Sampling Boundaries of the Study are as follows:

The sample collection is limited to the Creek Corridor. The investigation is limited to the surface sediments (0 to 6 inches below the sediment-water interface) because such sediments are readily bioavailable to fish and wildlife. Surface water and sediment samples need to be co-located.

Reference Areas:
A reference area for the fish and sediment sampling is required. Background sediment and fish data will be compared with existing OU2 sediment data and the new OU2 fish data using appropriate statistical tests.

The proposed reference area is a set of Mill ponds on the East Branch of Eighteenmile Creek as shown on Figure 11. These ponds are very similar to the Site as they were created by mills and have influence from the Barge Canal. A description of the mill ponds are found in the following link - http://nyhistoric.com/2013/11/mill-district-royalton/. Both ponds are rather large and appear to provide plenty of habitats for common fish species such as bass, sunfish, carp, and bullhead. There is an unpaved access ramp to the north pond and the south pond is nearly level with the road. Reviews of historical records indicate there is no source of contamination associated with the historical mills.

Upson Park Soil Samples
Elevated total PCBs were detected in surface and near-surface soil samples collected during multiple previous investigations from the northern portion of the Upson Park property, between the upper stream bank area and the driveway to the Park. Data gaps for Upson Park soil samples are listed below.

- Insufficient soil sample data are available to delineate the PCB 'hot spot' located between the western stream bank and the Park driveway in the northern portion of the Upson Park property. Additional sampling is recommended to define the nature and extent of contamination (see Figure 12).

Soil DQOs include the following:

- Determine the vertical and horizontal extent and concentration of total PCB contamination between the western stream bank and the Park driveway in the northern portion of the Upson Park property.

Soil Boundaries of the Study are as follows:

The area where additional delineation is recommended is bounded by the Upson Park driveway to the north and west, the creek bank to the east and the Upson Park parking area to the south. The Upson Park driveway is covered in gravel and stone and will not be sampled. The area between the current sample and the location of the historical samples with high PCBs is considered a boundary (see Figure 12).

Terrestrial Sampling

To support the Baseline Ecological Risk Assessment (BERA), vegetation, earthworms, and small mammals may be required to assess exposure pathways. Biota samples should be collocated with new soil samples so that site-specific biota soil accumulation factors can be developed for the site. The specific samples and parameters will be determined once the Screening Level Ecological Risk Assessment (SLERA) is complete.

Terrestrial DQOs include the following:

- Evaluate potential exposure pathways as determined by the SLERA.
- There are no risk screening values for mammals but % lipids analysis is required in order to assess bioaccumulation factors. Quantitation limit goals listed for soil in Worksheet 15 are considered acceptable for biota tissue.

Terrestrial Boundaries of the Study are as follows:

- Creek banks and wooded area along the Creek Corridor site.
- An appropriate reference location.

Table 12r Measurement Performance Criteria AVS/SEM

Matrix: Sediment
Analytical Group or Method: AVS/SEM – SW-846 9034 and 6010B and 7470
Concentration Level: Low

Data Quality Indicator (DQI)	QC sample or measurement performance activity	Measurement Performance Criteria
Overall Precision	Field Duplicates	RPD ≤ 40% for solids when analytes are detected in both samples ≥ sample-specific CRQL
Analytical Precision (laboratory)	Field Duplicates, MS/MSD	RPD ≤ 40% Field Duplicate AVS RPD ≤ 20% MS/MSD - TestAmerica Pittsburgh SOP No. PT-WC-008 Rev 5, Section 9.1 for Criteria SEM RPD ≤20% MS/MSD – TestAmerica Pittsburgh SOP No. PT-MT-001 Rev 14, Section 9.2 and SOP PT-MT-005 Rev 14, Section 9.0 for Criteria
Analytical Accuracy/Bias (matrix interference)	LCS	AVS Percent Recovery 85-115% - TestAmerica Pittsburgh SOP No, PT-WC-008 Rev 5, Section 9.1 for Criteria SEM Percent Recovery 80-120% - TestAmerica Pittsburgh SOP No. PT-MT-001 Rev 14, Section 9.2 and SOP PT-MT-005 Rev 14, Section 9.0- for Criteria
Analytical Accuracy/Bias (matrix interference)	MS/MSD	AVS Percent Recovery 75-125% - TestAmerica Pittsburgh SOP No, PT-WC-008 Rev 5, Section 9.1 for Criteria SEM Percent Recovery 75-125% - TestAmerica Pittsburgh SOP No. PT-MT-001 Rev 14, Section 9.2 and SOP PT-MT-005 Rev 14, Section 9.0- for Criteria
Analytical accuracy/bias (contamination)	Method Blanks	No target analyte concentrations >CRQL
Overall accuracy/bias (contamination)	Equipment Blanks	No target analyte concentrations >CRQL
Sensitivity	Annual MDL study	MDLs at or below method guidelines
Completeness	See Worksheet #34	See Worksheet #34

Table 12s Measurement Performance Criteria – Toxicity Testing

Matrix: Sediment
Analytical Group or Method: EPA 100.4 and 100.5
Concentration Level: Low

Data Quality Indicator (DQI)	QC sample or measurement performance activity	Measurement Performance Criteria
Analytical Precision and Accuracy/Bias (laboratory)	See method for controls and testing	Test Method 100.4: Hyalella azteca 28-day (chronic) Test for Measuring the Effects of Sediment-associated Contaminants on Survival, Growth, and Reproduction • Test Method 100.5: 20-day Test for Measuring the Effects of Sediment associated Contaminants on Chironomus tentans (midge)
Analytical accuracy/bias (contamination)	Laboratory controls	Acceptable water, test conditions, organisms. Reference toxicant testing.
Sensitivity	Test Length – Method 100.4	Laboratory will limit the test to 20 days for Chironomus and 28 days for Hyalella to measure Chronic Endpoints but not reproduction.
Completeness	See Worksheet #34	See Worksheet #34

Table 12t Measurement Performance Criteria – Toxicity Testing

Matrix: Surface Water
Analytical Group or Method: EPA 1000.0 and 1000.2
Concentration Level: Low

Data Quality Indicator (DQI)	QC sample or measurement performance activity	Measurement Performance Criteria
Analytical Precision and Accuracy/Bias (laboratory)	See method for controls and testing	Test Method 1000.1: Fathead Minnow, Pimephales promelas, Larval Survival and Growth Test Method Test Method 1002.0: Daphnid, Ceriodaphnia dubia, Survival and Reproduction Test
Analytical accuracy/bias (contamination)	Laboratory controls	Reference toxicant testing with control charts for each combination of toxicant, test species, test condition, and endpoint.
Sensitivity		
Completeness	See Worksheet #34	See Worksheet #34

QAPP Worksheet #14 & 16a – Project Tasks and Schedule

The project activities that will be performed during Spring 2015 are listed below. A master schedule will be maintained by the LATA Team and updated in monthly progress reports.

Table 14 & 16a Project Tasks and Schedule – Spring 2015

Activity	Responsible Party	Planned Start Date	Planned Completion Date	Deliverable(s)	Deliverable Due Date
QAPP Update	LATA Team	November 2014	November 2014	Supplemental QAPP Worksheets	November 17, 2014
Environmental Sampling - Fish, GW, soils, and Sed/SW	LATA Team	Mon 5/18/15	Fri 5/22/15	Field Logbooks and Daily Reports, Scribe Data	Daily
Environmental Sampling - Flintkote Borings	LATA Team	Samples will be collected when building is demolished but before backfill – Estimated in Late May 2015	Late May 2015	Field Logbooks and Daily Reports, Survey Data Points	Daily
Terrestrial Sampling	LATA Team	June 2015	June 2015	Field Logbooks and Daily Reports, Scribe Data	Daily
Routine Sample Analysis Non-routine Sample Analysis	Designated EPA Laboratory Subcontract Laboratories	May 2015	July 2015	Data Package in PDF format	14 days from sample receipt (same day or 48 hours for quick turn preliminary analyses)
Sample Analysis Validation	EPA or LATA Team for Subcontract Laboratories	May 2015	July 2015	Data Validation Report	June 30, 2015
Data Evaluation	LATA Team	May 2015	July 2015	Data Evaluation Report	July 31, 2015

Table 15o Reference Limits AVS/SEM

Matrix: Sediment
Analytical Method: EPA-182-R-91-100, and SW-846 9034, 6010B, 7470A
Concentration level: Low

Analyte	CAS No.	Project Action Limit (PAL) (mg/kg)[1]	PAL Reference	Reporting Limit	Reporting Limit Units
Metals (ICP)	**6010**				
Cadmium SEM	7440-43-9			0.125	mg/Kg
Silver SEM	7440-22-4			0.125	mg/Kg
Copper SEM	7440-50-8			0.625	mg/Kg
Lead SEM	7439-92-1			0.250	mg/Kg
Arsenic SEM	7440-38-2			0.250	mg/Kg
Nickel SEM	7440-02-0			1.00	mg/Kg
Zinc SEM	7440-66-6			2.50	mg/Kg
Chromium SEM	7440-47-3			0.125	mg/Kg
Cadmium SEM	7440-43-9			0.125	mg/Kg
Mercury (CVAA)	**7470A**				
Mercury (CVAA)	7439-97-6			0.000500	mg/Kg
Acid Volatile Sulfides (AVS)	18496-25-8			30.0	mg/Kg
Metals, Simultaneously Extracted Metals (SEM)					
SEM/AVS Ratio	STL00343			0.00100	NONE
Silver SEM	7440-22-4			0.00116	umol/g
Cadmium SEM	7440-43-9			0.00111	umol/g
Arsenic SEM	7440-38-2			0.00334	umol/g
Copper SEM	7440-50-8			0.00984	umol/g
Nickel SEM	7440-02-0			0.0170	umol/g
Chromium SEM	7440-47-3			0.00240	umol/g
Lead SEM	7439-92-1			0.000724	umol/g
SEM Summary	STL00344			0.00100	umol/g
Zinc SEM	7440-66-6			0.0382	umol/g
Mercury SEM	7439-97-6			0.0000623	umol/g
Acid Volatile Sulfides (AVS)	Acid Volatile Sulfides (AVS)			0.499	umol/g

Table 15o Reference Limits AVS/SEM

Matrix: Sediment
Analytical Method: EPA-182-R-91-100, and SW-846 9034, 6010B, 7470A
Concentration level: Low

Analyte	CAS No.	Project Action Limit (PAL) (mg/kg)[1]	PAL Reference	Reporting Limit	Reporting Limit Units

1. There are no applicable reference limits. Data will be evaluated based on . U.S. EPA, 2005. Procedures for the Derivation of Equilibrium Partitioning Sediment Benchmarks (ESBs) for the Protection of Benthic Organisms: Metals Mixtures (Cadmium, Copper, Lead, Nickel, Silver, Zinc). U.S. Environmental Protection Agency, EPA-600-R-02-011, January 2005).

Key
COC – Compounds of Concern
NA – Not Available
ND – Not Detected
* Guidance Value

QAPP Worksheet #17a – Sampling Design and Rationale

This worksheet presents the sampling design and rationale for specific sampling activities to be conducted in spring 2015. Samples designated to fill data gaps for the baseline ecological risk assessment (BERA) are expected to be better defined following the screening level risk assessment (SLERA). In addition, biological sampling is not recommended for colder weather and should be completed during warmer months with greater biological activity.

Samples will be analyzed for the Target Compound list/Target Analytical List (TCL/TAL) parameters for RAS under the CLP program. In addition, samples will be analyzed for non-RAS parameters under the CLP program including Multi-Media, Multi-Concentration Dioxins and Furans Analysis (DLM02.2) and Multi-Media, Multi-Concentration Chlorinated Biphenyl (CB) Congeners (CBC01.2). One sample from each of the major areas sampled during the event will be analyzed for dioxin/furan, hexavalent chromium, and PCBs congeners in order to have a few representative samples with a TCL/TAL analyses for screening purposes. For analysis of PCBs in surface water, the samples should be analyzed with low-level PCB congener analysis to maintain consistency with historical data and achieve lower detection limits.

Flintkote Soil Borings
For the soil borings collected at Flintkote building following the building demolition samples will be collected based on the conditions present. The area is planned to be backfilled following sampling, therefore analysis of samples for additional parameters for risk assessment is not considered necessary because the surface area will not be exposed.

Fish Samples
Fish (forage and edible) will be collected in the Creek corridor using electroshocking and netting techniques. The target fish species are expected to be juvenile sunfish (Lepomis spp.) and adult largemouth bass (Micropterus salmoides). Both species are expected to be plentiful in OU2 based on historical sampling in other areas of the Creek. For largemouth bass, skin-on fillet samples will be collected following NYSDEC protocols for use in the human health risk assessment. Whole-body composite samples of juvenile sunfish will be collected for use in the ecological risk assessment.

In order to allow statistical comparison between the site and reference location, field crews will attempt to collect 10 samples from each location. The number of samples will depend on the field conditions as described in Worksheet 18a. For dioxins, collection of only one sample from each area will not allow for statistical comparison.

Sediment and Surface Water Samples
Surface sediment (0 to 6 inches beneath the sediment water interface) and surface water samples will be collected in the Creek channel at three sample locations and one reference area location for both chemical parameters and toxicity testing. Sediment samples will be collected in shallow water using a hand-held Ponar sampler (multiple grabs for significant volume) and surface water will be collected using bottle direct- fill methods. Chemistry and toxicity samples will be collocated. Surface water parameters (pH, temperature, and specific conductivity) will also be monitored at each location with a Horiba U-22 multi-parameter probe or equivalent. All chemical and toxicity samples will be co-located. The surface water and sediment chemistry will be analyzed for a full suite of TCL/TAL parameters to provide additional data for human health and ecological risk assessment.

Sample locations are shown on Figures 10 and 10a. Based on existing data, average sediment concentrations for PCBs behind Clinton Street Dam are the lowest and increase to the Millrace

area with the highest concentrations. Average sediment concentrations for lead are the highest behind Clinton Street Dam and decrease to the Millrace area with lower concentrations.

Sediment samples for chemical analysis will also be collected from a reference location for statistical comparison to existing OU2 sediment data. Background samples will be analyzed for the same contaminants as site samples. Site and reference samples will be compared using appropriate statistical methods.

Upson Park Soil Samples

To delineate vertical and horizontal extent, as well as concentration of PCB contamination in soils collected from the northern portion of Upson Park, additional surface soil (0 to 6 inches) and near-surface soil (6 to 24 inches) samples will be collected from four (4) locations (see Figure 12). One soil sample location will be established approximately 25 feet north, south and east of OU2-SS09. One soil sample location will be established between OU2 SS09 and DEC Upson-2 to determine if there is contamination between the two highest PCB concentrations. Figure 12 shows PCB concentrations from the previous samples.

At each location, samples will be collected from the ground surface to 0.5 feet below ground surface (BGS) and 0.5 feet BGS to 2 feet BGS to be consistent with previous sample collection methods. In addition, one sample from 2 feet BGS to 3 feet BGS will be collected if possible with the hand auger to assess whether PCB concentrations increase or decrease with depth. Samples will be collected using dedicated stainless steel bowls and spoons. One half-day has been estimated for completion of the soil sampling in this area.

Terrestrial Sampling

The specific samples will be determined once the SLERA is complete. For estimating purposes, it is assumed that samples will be collected at three locations per property plus three locations at a suitable reference area for a total of 15 locations. Surface soil (0 to 0.5 feet bgs) and near surface soil (0.5 to 2 feet bgs) will be collected at same locations. Samples will be analyzed for a full suite of parameters to support both human health and ecological risk assessment. The approximate sample locations will be determined after the SLERA. Although samples will be collected on a specific property, the available habitat along the Creekside is very similar and the samples can be assessed as a group as representative of the entire OU2 for evaluation of general environmental contaminants, such as pesticides and other organics.

QAPP Worksheet #18a – Sampling Locations and Methods

The sampling locations, methods, and number of samples are presented on Table 18a and Figure 10. The proposed reference sediment location is shown on Figure 11. Sampling SOPs are listed on Worksheet #21a.

All samples for the Spring 2015 sampling phase will be collected with dedicated or disposal equipment that does not require decontamination except for the ponar sampling used to collected sediment samples. One equipment blank for ponar will be collected. If field conditions necessitate changing the sampling procedures requiring decontamination then rinsates will be collected.

Fish Samples

Each of the areas will have a similar level of effort for electrofishing and seine activity (e.g. electroshock for 15 minute durations or collect three beach seine hauls). Actual sampling techniques will be dependent upon results of the fish collection effort and the effectiveness of various techniques and suitability of habitat. If unforeseen circumstances arise that impact this scope of work, we will discuss this with EPA and determine an appropriate course of action and the associated cost implications.

Sampling will be conducted with a boat-mounted Smith-Root electrofishing unit in near-shore and shallow areas. Collection efforts for each electroshocking run will be approximately 15 minutes. Overall reach lengths will be determined in the field, but will fall likely within the range of 500 – 1,000 feet. Fish immobilized during each electrofishing run will be dip-netted and put into aerated live wells for processing.

We also may employ a seine at each location to augment the sampling efforts in an attempt to collect smaller fish that may be missed by the electroshocking. Seine methods will follow standard protocols and be performed in a reproducible manner based on the individual site constraints at each location.

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 on the sample form included in Appendix A. Identification of specimens will be to the lowest practicable taxonomic level using 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.

No voucher specimens will be maintained 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 analysis 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 is similar. In such circumstances, a sub-sample of 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.

Targeted fish sizes will match the legal or typical catch size to minimize size and age variation among the sample set. The state legal minimum for bass is 12 inches, as such bass will be targeted for collection in the 15 to 20 inch size range, with an alternative range of 12 to 24 inches, based on the number actually caught at each location. All bass will be held alive in the 12 to 24 inch range, along with surrogate fish species (carp, bullhead, smallmouth bass, pumpkinseed, rock bass, and yellow perch) if no or very few bass are caught. If four bass are collected in the 15 to 20 size range, then they will be retained for analysis and the remaining bass and surrogate species in the live well will be released. If some but not all of the bass collected are in the 15 to 20 inch size range, the field team leader will make the decision on which fish to retain, favoring largemouth bass first in the targeted range and second in the expanded size range. If no bass are collected then the team will continue to electrofish for one more hour, move to the next site and plan to return later to the site if time permits within the budgeted four-day timeframe.

For forage fish, whole-body composite samples of juvenile sunfish (approximately 4 inches total length) will be collected for use in the ecological risk assessment. If sunfish are not found, shiner or other similar forage fish will be collected. The target number of individuals per sample is 10 and the target number of samples is 10.

As per above methods, all fish will be measured and weighed. 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. Sport fish will then be prepared for analysis at the laboratory for analysis (if possible) by excising standard fillets (scales removed) and reserving the remaining carcass. The weight of the standard fillets and the carcass will be individually weighed and the individual weights recorded as the total fish 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 laboratory cannot fillet the fish then the procedure will be performed in the field on a clean surface.

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 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.

Sediment and Surface Water Samples

Surface sediment samples (0-6 inches) will be collected from the surface with a Ponar or Ekman dredge and surface water samples will be collected by the direct fill method from the mid-depth of the water column. The team will use the methodology described below:

- Navigate to sample location and determine the water depth to top of sediment. Collect the water sample first at a mid-depth.
- Collect sediment using a Ponar or Ekman dredge. Place sediment from the Ponar in a dedicated stainless-steel bowl for chemical testing and plastic bucket for toxicity testing (depending on the volume requirements for the testing to be performed). The field geologist will then record a description of the sediment, Ponar recovery, water depth, and visual/olfactory observations in the field notebook and/or field forms.
- Photograph every sediment sample and/or sampling location. Photographs of the ponar operation will also be collected throughout the field program. For each photograph, record the sample time (time photo taken), date, photograph compass orientation, and description. Record this data in the logbook.
- Record the general sample location coordinate using a hand held GPS unit. It is anticipated that multiple sediment grabs will be samples are required to obtain adequate volume for toxicity testing.

Once all the necessary information has been recorded and samples collected for chemical analysis, the remaining sediment will be placed in a large plastic bucket and thoroughly homogenized. Stones and debris will be removed.

Table 18a Sample Summary Spring 2015		**Number of Samples**					**Number of Samples per Method**						
Sample Media	**Notes**	**No. of Locations**	**No. of Background Locations**	**No. of Samples**	**No. of QA/QC Samples**	**Total**	**PCBs/ Pest/ SVOCs**	**VOCS**	**Inorganic**	**Dioxin/ Furan DLM02.2**	**CB Congeners CBC01.2**	**Hexavalent Chromium**	**TCLP**
Ground water	6 Wells in and upgradient of VOCs detected south of the creek. Data to characterize upgradient VOC sources and provide data for HHRA. Round Two	6		6	1		7	7	7			1	
Subsurface soil	Ten borings after the Flintkote building is removed. Depths: 0-6", 1-2', and one additional depth selected in field based on staining. Samples for both characterization of PCB contamination and risk assessment purposes.	10		30	4		34	34	34	1	1	0	
Sediment	Sediment collected from upstream and downstream of Clinton Street dam, and from millrace adjacent to former Flintkote property and Reference Area	3	1	4	1	5	5	5	5	1	1	1 Also 5 TOC	
Surface Water	Surface Water collected from upstream and downstream of Clinton Street dam, and from millrace adjacent to former Flintkote property and Reference Area	3	1	4	2 Rinsate Blank of Ponar	6	6	6	6		4	2	
Sediment	Reference – Sediment collected from East Branch Reference location	0	9	9	0	9	9	9	9	1	1	Also 9 TOC	
Fish Tissue	Sport Fish - Largemouth bass (or carp or bullhead) collected from OU2 and Reference Location	1	1	20		20	20		20	2	2	Also 20 %Lipids	

Table 18a Sample Summary Spring 2015		**Number of Samples**					**Number of Samples per Method**						
Sample Media	**Notes**	**No. of Locations**	**No. of Background Locations**	**No. of Samples**	**No. of QA/QC Samples**	**Total**	**PCBs/ Pest/ SVOCs**	**VOCS**	**Inorganic**	**Dioxin/ Furan DLM02.2**	**CB Congeners CBC01.2**	**Hexavalent Chromium**	**TCLP**
Fish Tissue	Pumpkinseed (or other sunfish) collected from OU2 and Reference Location	1	1	20		20	20		20	2	2	Also 20 %Lipids	
Surface Soil	Additional surface soil collected from north portion Upson Park. Surface soil (0 to 0.5 feet) and near surface soil (0.5 to 2 feet and 2 to 3 feet).	4		12	2	14	14 (PCB Only)			1	1		
IDW solid	Soil cuttings; Toxicity characteristic leaching procedure (TCLP) parameters except herbicides, PCBs, corrosivity, and ignitibility	2		2		2	2 (PCB Only)						2
IDW liquid	Decon fluids and monitoring well purge water	1		1		1	1 (PCB Only)						1
	Totals												

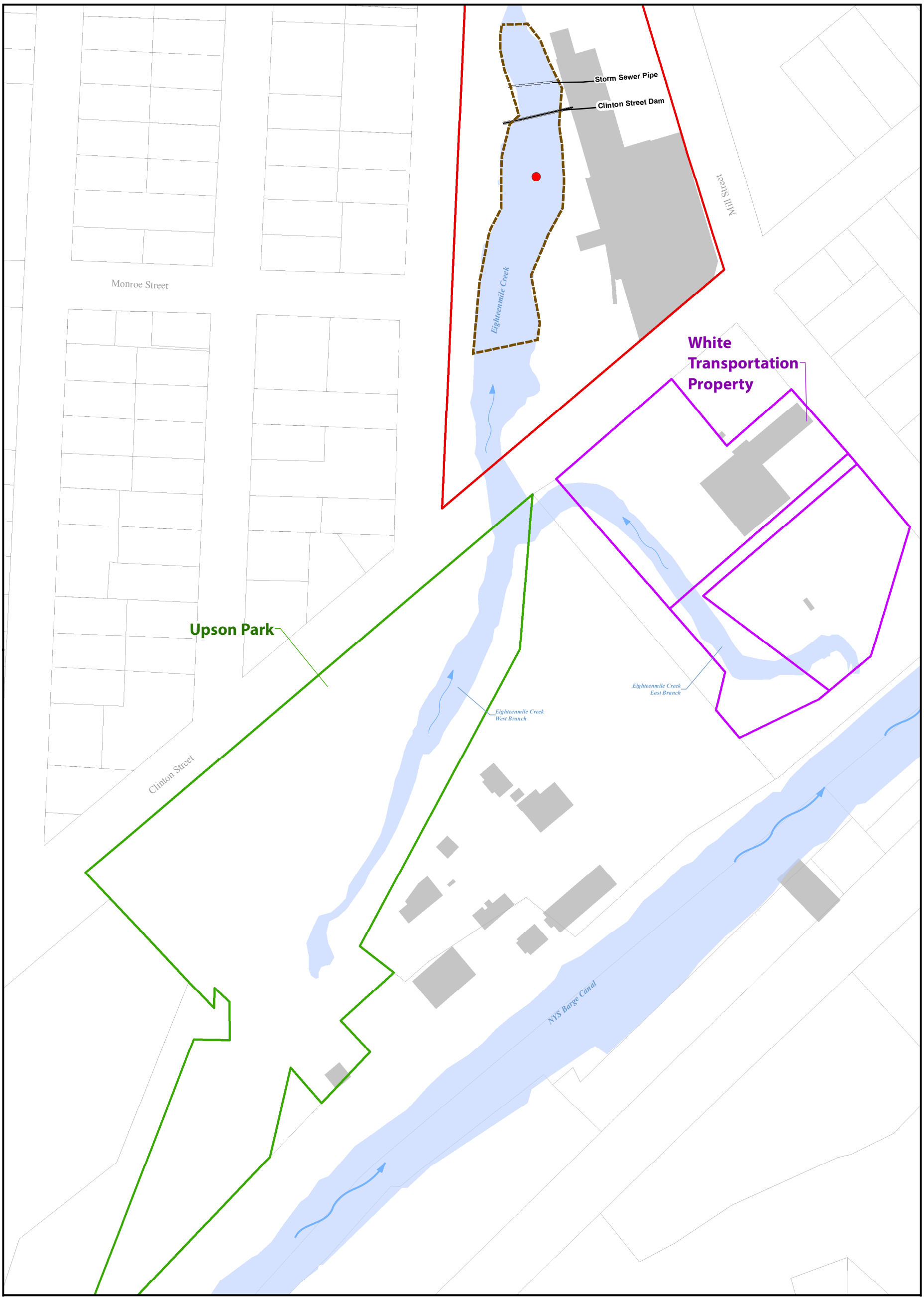


Figure 10. Eighteen Mile Creek OU 2
Proposed Fish, Sediment and Surface Water Sample Locations

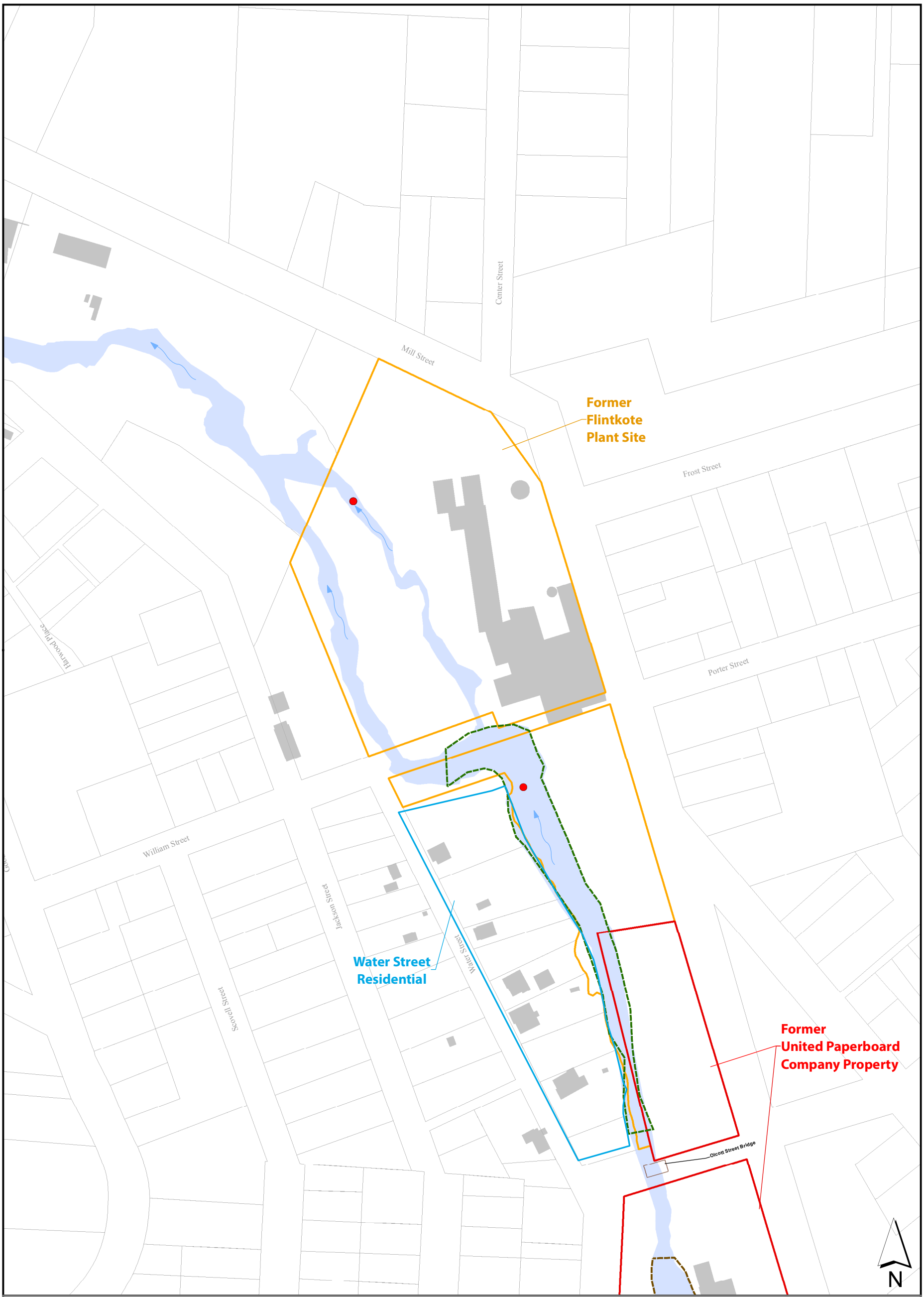


Figure 10a. Eighteen Mile Creek OU 2
Proposed Fish, Sediment and Surface Water Sample Locations

Document Path: L:\Buffalo\eighteenmile\Mxds\OU2\2015_April\Fig11_OU2_Reference_Sample_Locations.mxd Date: 4/27/2015

Niagara County

84.00-3-42.111

84.00-3-35.2

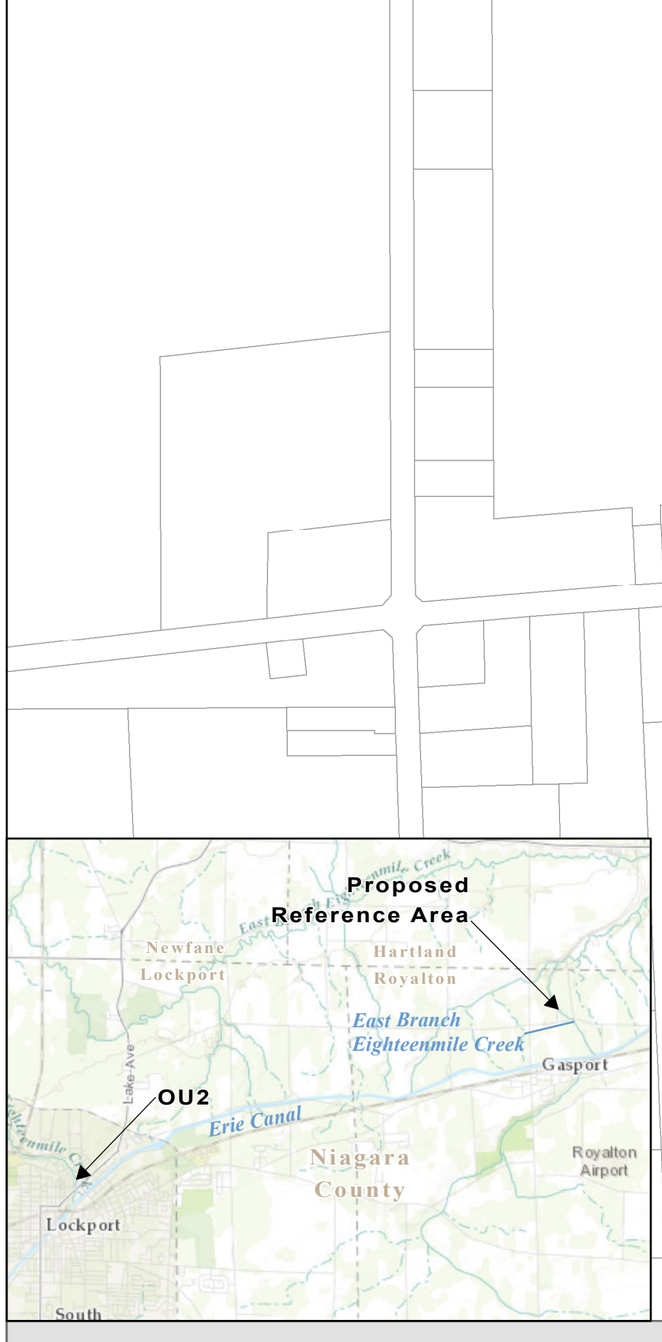
Quaker Rd

Slayton Settlement Rd

East Branch Eighteenmile Creek

Bolton Rd

Erie Canal



Background Sediment (Locations are Approximate)

Proposed Forage Fish and Sport Fish Area

Tax Parcels (2015)

Water Feature

N

0 250 500 1,000 Feet

Figure 11
Eighteen Mile Creek OU 2 Proposed Fish, Sediment and Surface Water Reference Sample Locations

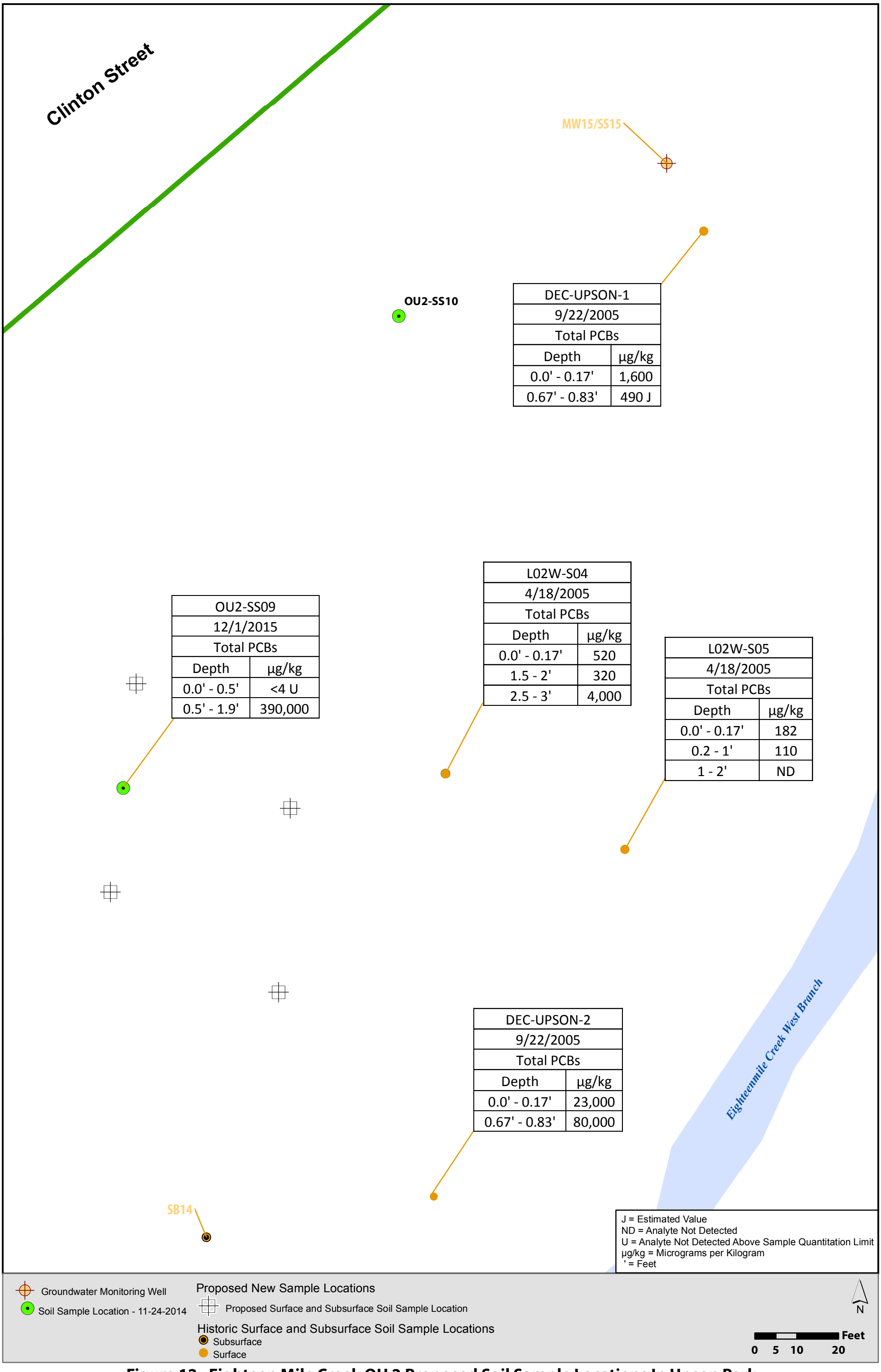


Figure 12. Eighteen Mile Creek OU 2 Proposed Soil Sample Locations In Upson Park

Laboratory: TestAmerica Pittsburgh
List any required accreditations/certifications: NYSDOH ELAP

Back-up Laboratory: N/A
Sample Delivery Method: Shipping Overnight

Table 19 & 30a Sample Containers, Preservation, and Hold Times – Subcontract Laboratory

Matrix	Analytical Group	Analytical / Preparation Method SOP Reference[1]	Containers (number, size, and type)	Sample volume[3] (units)	Preservation Requirements	Maximum Holding Time[2] (preparation / analysis)	Data Package Turnaround Time[4]
Groundwater and Surface Water	Hexavalent Chromium	SW-846 7196A	(1) 250 mL HDPE bottle	125 mL	Cool to 4°C	24 hours	28 days
Soil and Sediment	Hexavalent Chromium	SW-846 7196A (SW-846 3060A prep)	(1) 4 oz. glass jar w/Teflon lined cap	30 grams	Cool to 4°C	30 days	28 days
Sediment	AVS/SEM	SW-846 9034, 6010B, 7470A (EPA-182-R-91-100 prep)	(1) 4 oz. glass jar w/Teflon lined cap	30 grams	Cool to 4°C	(14 days for prep) AVS 7 days prep to analysis, SEM Hg 28 days to analysis, all other metals 6 months	28 days

[1] Refer to the Analytical SOP References table (Worksheet #23).
[2] Maximum holding time is calculated from the time the sample is collected to the time the sample is prepared/extracted unless otherwise specified.
[3] The minimum sample size is based on analysis allowing for sufficient sample for reanalysis. Additional volume is needed for the laboratory Matrix Spike/Matrix Spike Duplicate sample analysis.
[4] 28 day turnaround time for laboratory results based on AVS/SEM requirements.

Key:
DI = Deionized.
HDPE = High density Polyethylene.

Laboratory: Great Lakes Environmental Center, Inc. (GLEC)
List any required accreditations/certifications: NELAC

Back-up Laboratory: N/A
Sample Delivery Method: Shipping Overnight

Table 19 & 30b Sample Containers, Preservation, and Hold Times – Toxicological Sampling

Matrix	Analytical Group	Analytical / Preparation Method SOP Reference[1]	Containers (number, size, and type)	Sample volume[3] (units)	Preservation Requirements	Maximum Holding Time[2] (preparation / analysis)	Data Package Turnaround Time[4]
Surface water	Toxicity	Test Method 1000.1: Fathead Minnow, Pimephales promelas, Larval Survival and Growth Test Method	4 x 2.5-gal cubitainer of water	7 gallon	Cool to 4°C	36 hours	35 days
Surface water	Toxicity	Test Method 1002.0: Daphnid, Ceriodaphnia dubia, Survival and Reproduction Test	With Method 1000.1		Cool to 4°C	36 hours	35 days
Sediment	Toxicity	Test Method 100.4: Hyalella azteca 42-day (chronic) Test for Measuring the Effects of Sediment-associated Contaminants on Survival, Growth, and Reproduction •	1 x 2.5-gal pail of sediment	1 gallon	Cool to 4°C	8 weeks	35 days
Sediment	Toxicity	Test Method 100.5: 20-day Test for Measuring the Effects of Sediment associated Contaminants on Chironomus tentans	With Method 100.4		Cool to 4°C	8 weeks	35 days

[1] Analytical SOP References will be determined when laboratory is selected.
[2] Maximum holding time is calculated from the time the sample is collected to the time the sample is prepared/extracted unless otherwise specified. Samples for surface water must be shipped overnight delivery.
[3] The minimum sample size will be determined based on laboratory SOPs..
[4] 35 day turnaround time for laboratory results assumes 28-days for testing and 7 days for the report.

Table 20a Field Quality Control Summary –Spring 2015

Sample Matrix	Analyte/ Analytical Group	Conc Level	Analytical and Preparation SOP Reference	No. of Sampling Locations	No. of Field Samples	No. of Reference Samples	No. of Field Duplicate Sample Pairs	No. of MS/MSDs	No. of Field Blanks[1]	No. of Equipment Rinsate Blanks[1]	No. of Trip Blanks	Total Number of Analyses
Groundwater (low-flow)	PCBs Pesticides SVOCs	Low	CLP Routine - Organic SOM01.2	6	6	0	1 per 10 samples or 10%	1 per 20 samples up to one week or 5%	0	0	0	8
Groundwater (low-flow)	VOCs	Trace	CLP Routine - Organic SOM01.2	6	6	0	1 per 10 samples or 10%	1 per 20 samples up to one week or 5%	0	0	One per cooler	9
Groundwater (low-flow)	Inorganics	Low	CLP Routine - Inorganic ISM01.3	6	6	0	1 per 10 samples or 10%	1 per 20 samples up to one week or 5%	0	0	0	8
Groundwater (low-flow)	Hexavalent Chromium	Low	SW-846 7196A	1	1	0	0	0	0	0	0	1
Upson Park Hand Augar Soils (three depths)	PCBs	Low	CLP Routine - Organic SOM01.2	4	12	0	1 per 20 samples or 5%	1 per 20 samples up to one week or 5%	0	0	0	14
Upson Park Hand Augar Soils (Surface)	Dioxin/ Furan	Low	CLP Non-Routine - Dioxin/Furan DLM02.2	1	1	0	0	0	0	0	0	1
Upson Park Hand Augar Soils (Surface)	CB Congeners	Low	CLP Non-Routine - CB Congeners CBC01.2	1	1	0	0	0	0	0	0	1
Fish Tissue – Whole Body	PCBs Pesticides SVOCs	Low	CLP Routine - Organic SOM01.2	1	10	10	0	0	0	0	0	20
Fish Tissue – Whole Body	VOCs	Low	CLP Routine - Organic SOM01.2	0	0	0	0	0	0	0	0	0
Fish Tissue – Whole Body	Inorganics	Low	CLP Routine - Inorganic ISM01.3	1	10	10	0	0	0	0	0	20
Fish Tissue – Whole Body	Percent Lipids	Low		1	10	10	0	0	0	0	0	20

Table 20a Field Quality Control Summary –Spring 2015

Sample Matrix	Analyte/ Analytical Group	Conc Level	Analytical and Preparation SOP Reference	No. of Sampling Locations	No. of Field Samples	No. of Reference Samples	No. of Field Duplicate Sample Pairs	No. of MS/MSDs	No. of Field Blanks[1]	No. of Equipment Rinsate Blanks[1]	No. of Trip Blanks	Total Number of Analyses
Fish Tissue – Whole Body	Dioxin/ Furan	Low	CLP Non-Routine - Dioxin/Furan DLM02.2	1	1	1	0	0	0	0	0	2
Fish Tissue – Whole Body	CB Congeners	Low	CLP Non-Routine - CB Congeners CBC01.2	1	1	1	0	0	0	0	0	2
Fish Tissue - Fillet	PCBs Pesticides SVOCs	Low	CLP Routine - Organic SOM01.2	1	10	10	0	0	0	0	0	20
Fish Tissue - Fillet	VOCs	Low	CLP Routine - Organic SOM01.2	0	0	0	0	0	0	0	0	0
Fish Tissue - Fillet	Inorganics	Low	CLP Routine - Inorganic ISM01.3	1	10	10	0	0	0	0	0	20
Fish Tissue - Fillet	Percent Lipids	Low		1	10	10	0	0	0	0	0	20
Fish Tissue - Fillet	Dioxin/ Furan	Low	CLP Non-Routine - Dioxin/Furan DLM02.2	1	1	1	0	0	0	0	0	2
Fish Tissue - Fillet	CB Congeners	Low	CLP Non-Routine - CB Congeners CBC01.2	1	1	1	0	0	0	0	0	2
Sediment	PCBs Pesticides SVOCs	Low	CLP Routine - Organic SOM01.2	3	3	10	0	1 per 20 samples up to one week or 5%	1 per 20 samples up to one week or 5%	0	0	15

Table 20a Field Quality Control Summary –Spring 2015

Sample Matrix	Analyte/ Analytical Group	Conc Level	Analytical and Preparation SOP Reference	No. of Sampling Locations	No. of Field Samples	No. of Reference Samples	No. of Field Duplicate Sample Pairs	No. of MS/MSDs	No. of Field Blanks[1]	No. of Equipment Rinsate Blanks[1]	No. of Trip Blanks	Total Number of Analyses
Sediment	VOCs	Low	CLP Routine - Organic SOM01.2	3	3	10	0	1 per 20 samples up to one week or 5%	1 per 20 samples up to one week or 5%	0	One per cooler	15
Sediment	Inorganics	Low	CLP Routine - Inorganic ISM01.3	3	3	10	0	1 per 20 samples up to one week or 5%	1 per 20 samples up to one week or 5%	0	0	15
Sediment	Hexavalent Chromium	Low	SW-846 7196A	1	1	1	0	0	0	0	0	2
Sediment	Total Organic Carbon		Lloyd Kahn	3	3	10	0	0	0	0	0	15
Sediment	Dioxin/ Furan	Low	CLP Non-Routine - Dioxin/Furan DLM02.2	1	1	1	0	0	0	0	0	2
Sediment	CB Congeners	Low	CLP Non-Routine - CB Congeners CBC01.2	1	1	1	0	0	0	0	0	2
Surface Water	CB Congeners	Low	CLP Non-Routine - CB Congeners CBC01.2	3	3	1	0	0	0	0	0	4
Surface Water	Pesticides SVOCs	Low	CLP Routine - Organic SOM01.2	3	3	1	0	1 per 20 samples up to one week or 5%	0	0	0	5
Surface Water	VOCs	Low	CLP Routine - Organic SOM01.2	3	3	1	0	1 per 20 samples up to one week or 5%	0	0	One per cooler	6
Surface Water	Inorganics	Low	CLP Routine - Inorganic ISM01.3	3	3	1	0	1 per 20 samples up to one week or 5%	0	0	0	5

Table 20a Field Quality Control Summary –Spring 2015

Sample Matrix	Analyte/ Analytical Group	Conc Level	Analytical and Preparation SOP Reference	No. of Sampling Locations	No. of Field Samples	No. of Reference Samples	No. of Field Duplicate Sample Pairs	No. of MS/MSDs	No. of Field Blanks[1]	No. of Equipment Rinsate Blanks[1]	No. of Trip Blanks	Total Number of Analyses
Sediment	TOX		Method 100.4 and 100.5	3	3	1						4
Surface Water	TOX		Method 1000.0 and 1000.2	3	3	1						4
Subsurface Soil (three depths)	PCBs Pesticides SVOCs	Low	CLP Routine - Organic SOM01.2	10	30	0	20 or 5%	1 per 20 samples up to one week or 5%	0	0	0	34
Subsurface Soil (three depths)	VOCs	Low	CLP Routine - Organic SOM01.2	10	30	0	1 per 20 samples or 5%	1 per 20 samples up to one week or 5%	0	0	2	36
Subsurface Soil (three depths)	Inorganics	Low	CLP Routine - Inorganic ISM01.3	10	30	0	1 per 20 samples or 5%	1 per 20 samples up to one week or 5%	0	0	0	34
Subsurface Soil (Surface depth)	Dioxin/ Furan	Low	CLP Non-Routine - Dioxin/Furan DLM02.2	1	1	0	0	0	0	0	0	1
Subsurface Soil (Surface depth)	CB Congeners	Low	CLP Non-Routine - CB Congeners CBC01.2	1	1	0	0	0	0	0	0	1
IDW (Soil/Water)	TCLP and PCBs	Low/ Med	SW-846 1311 and 8082	3*	3*	0	0	0	0	0	0	3*

[1] Equipment blanks are only required when non-dedicated or disposal equipment is used. Rinsate blank for the Ponar sediment sampling is counted with surface water samples in Table 18a.

* Estimated

Note 1 – Field QC samples will not be collected for the IDW soil or water samples.

QAPP Worksheet #21 – Field SOPs

SOPs for E & E field personnel are stored on E & E's intranet site.

Table 21 Project Sampling SOP References

SOP or Reference Number	Title, Revision Date and / or Number	Originating Organization	SOP Option or Equipment Type	Modified for Project Work? (Y/N)	Comments
EPA 1998	EPA Region II Groundwater Sampling Procedure – Low Stress (Low Flow) Purging and Sampling	EPA Region II	Horiba Model U-22 flow-through cell or equivalent	N	
ENV 3.7	Groundwater Well Sampling, Revision Date 6/15/2012	E & E		N	
ENV 3.13	Surface and Shallow Subsurface Soil Sampling, Revision Date 5/25/2012	E & E			
ENV 3.15	Sampling Equipment Decontamination, Revision Date 5/25/2012	E & E		N	
HS 5.3	Health and Safety on Drilling Rig Operations, Revision Date 5/1/2008	E & E		N	
GEO 4.8	Geologic Logging, Revision Date 3/1/1998	E & E		N	
GEO 4.10	Monitoring Well Installation, Revision Date 3/1/1998	E & E		N	
GEO 4.11	Well Development, Revision Date 4/2/2013	E & E		N	
ENV 3.26	Handling IDW, Revision Date 5/25/2012	E & E		N	
ENV 3.28	FISH SAMPLING	E & E		N	Follow NYSERDA 2008 for fillets
ENV 3.8	AQUATIC SEDIMENT SAMPLING	E & E		N	
ENV 3.12	SURFACE WATER SAMPLING	E & E		N	

Table 23b Analytical SOPs –Subcontract Laboratory

SOP #	Title, Date, and URL (if available)	Definitive or Screening Data	Matrix/Analytical Group	SOP Option or Equipment Type	‡Modified for Project? Y/N
PT-WC-015 Rev.:16 Effective Date: 3/1/2013	Chromium, Hexavalent (Colorimetric) Method(s): SW846 3060A / 7196A and Standard Method 3500-Cr B	Definitive	Chromium, Hexavalent	Spectrophotometer	N
PT-WC-008 Rev. 5 Effective Date 5/15/2014	Acid Volatile Sulfides (AVS) and Simultaneously Extracted Metals (SEM) in Sediment	Definitive	AVS	None	N
PT-MT-001 Rev. 14 Effective Date 07/11/2013	Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP)	Definitive	SEM	ICP	N
PT-MT-005 Rev. 14 Effective Date 04/24/2013	Preparation and Analysis of Mercury in Aqueous Samples by Cold Vapor Atomic Absorption	Definitive	SEM	CVAA	N
SED 7003	STANDARD OPERATING PROCEDURE FOR 28-DAY AND 42-DAY LIFE-CYCLE WHOLE SEDIMENT TOXICITY TESTS WITH Hyalella Azteca, January 2012, Appendix B	Definitive	Toxicity Testing		28 day
SED 7004	STANDARD OPERATING PROCEDURE FOR LONG TERM WHOLE SEDIMENT TOXICITY TESTS WITH Chironomus dilutus, January 2012, Appendix B	Definitive	Toxicity Testing		20 day
TOX 0025	STANDARD OPERATING PROCEDURE FOR STATIC-RENEWAL SHORT-TERM CHRONIC TOXICITY TESTS WITH Flathead Minnows, May 2011, Appendix B	Definitive	Toxicity Testing		N
TOX 0023	STANDARD OPERATING PROCEDURE FOR STATIC-RENEWAL SHORT-TERM CHRONIC EFFLUENT TOXICITY TESTS WITH Ceriodaphnia, May 2011, Appendix B	Definitive	Toxicity Testing		N

Table 24b Analytical Instrument Calibration –Subcontract Laboratory

Instrument	Calibration Procedure	Calibration Range	Frequency of Calibration	Acceptance Criteria	Corrective Action (CA)	Person Responsible for CA	SOP Reference
Spectrophotometer	See PT-WC-015 Rev.:16 (Appendix B)	Low	Every 15 samples	Acceptance limits are ±10 percent of the true value of the standard.	If CCV fails, correct problem then repeat CCV and reanalyze all samples since last successful calibration verification.	Laboratory Technician	PT-WC-015 Rev.:16
ICP	See SOP PT-MT-001 Rev. 14, Section 10.1	Low	Calibration Verification every 10 samples	Acceptance limits are ±10 percent of the true value of the standard.	If CCV fails, correct problem then repeat CCV and reanalyze all samples since last successful calibration verification.	Laboratory Technician	SOP PT-MT-001 Rev. 14, Section 10.1
CVAA	See SOP PT-MT-005 Rev. 14, Section 10.1	Low	Calibration Daily, Calibration Verification every 10 samples	ICAL linear correlation coefficient $\geq$0.995 Verification acceptance limits are ±10 percent of the true value of the standard.	If CCV fails, correct problem then repeat CCV and reanalyze all samples since last successful calibration verification.	Laboratory Technician	SOP PT-MT-005 Rev. 14, Section 10.1

Table 28a QC Sample Summary

Matrix: Sediment
Analytical Group: AVS/SEM
Analytical Method/SOP: SW-846 9034, 6010B, 7470A

QC Sample	Frequency/ Number	Method/SOP QC Acceptance Limits	Corrective Action	Title/position of person responsible for corrective action	Project-Specific MPC
Equipment blank	0	One-half the reporting limits.	Associated sample results are qualified non-detect if less than 5 times the blank level.	E & E Data Validation Chemist	No target compounds of concern detected in blanks in field batch.
Field Duplicate	0		Changes in field procedures should be implemented if a high degree of variability if found.	E & E Data Validation Chemist	RPD must be calculated as a comparison of measured concentrations. See Worksheet #12
MS/MSD (Lab QC)	1 set per batch.	75-125% R, RPD ≤ 20% for AVS and SEM	1) Evaluate the LCS. If LCS is within acceptance limits, report and narrate results.	Laboratory Analyst	The results of all MS/MSDS must be evaluated against the acceptance criteria for LCS
LCS	1 per batch	For AVS 85-115% recovery; For SEM 80-120% recovery	Reanalyze LCS; if still outside of criteria and LCSD is in control no action is required; if both the LCS and LCSD fail reprepare and reanalyze all associated samples.	Laboratory Analyst	For evaluation and acceptance criteria, see SW-846 methods 9034, 6010B and 7470A
Method Blanks	1 per batch	The concentrations shall not exceed the reporting limit.	Reanalyze MB, if still outside of criteria redigest and reanalyze MB in the batch; however if MB > RL and samples are non-detect or >10x the concentration of analyte in MB, NCM and no corrective action is required.	Laboratory Analyst	No target compounds of concern detected in blanks for preparation batch.

Analytical Group/Method:	**AVS/SEM**	**Toxicity Testing**
Data deliverable requirements:	Stage 3	Stage 3
Analytical specifications:	Worksheet 24	Worksheet 24
Measurement performance criteria:	Worksheet 12	Worksheet 12
Percent of data packages to be validated:	100%	100%
Percent of raw data reviewed:	0	10%
Percent of results to be recalculated:	0	10%
Validation procedure:	EPA Region 2 Data Validation SOPs	EPA Region 2 Data Validation SOPs
Validation code (*see attached table):	S2bVEM	S2bVEM
Electronic validation program/version:	EPA MEDD	EPA MEDD

QAPP Appendix A – Field and Data Documentation

The referenced SOPs for the field sampling are posted on E & E's intranet site.

Daily report is included in this appendix.

Table 1 Summary of Historical Data is included in this appendix.

Data validation checklist for hexavalent chromium is included in this appendix.

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 ____**

NEW YORK STATE DEPARTMENT OF ENVIRONMENTAL CONSERVATION
BUREAU OF HABITAT

FISH PREPARATION PROCEDURES FOR CONTAMINANT ANALYSIS

Background

New York State Department of Environmental Conservation (DEC) conducts studies requiring chemical analysis on fish tissues. Routine monitoring and surveillance studies develop data on contaminants in fish for several reasons:

1. To identify sources of environmental contamination

2. To identify the geographic extent of environmental contamination

3. To identify temporal trends of contaminants in fish and wildlife

4. To provide information regarding human consumption advisories

Chemical analyses of edible-fish flesh have been determined to be the most appropriate analyses for satisfying all of these objectives. The following methodology has been developed in order to standardize the tissues under analysis and to adequately represent the contaminant levels of fish flesh. The methodology is slightly modified from the U.S. Food and Drug Administration procedures. The portion of edible flesh analyzed will be referred to as the standard fillet unless otherwise noted. For some species, the procedure is modified as indicated below.

Procedures for Standard Filleting:

1. Remove scales from fish. Do not remove the skin.

2. Make a cut along the ventral midline of the fish from the vent to the base of the jaw.

3. Make diagonal cut from base of cranium following just behind gill to the ventral side just behind pectoral fin.

4. 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.

5. Score the skin and homogenize the entire fillet.

Four modifications of the standard fillet procedure are designed to account for variations in fish size or known preferred preparation-methods of the fish for human consumption.

1. Some fish are too small to fillet by the above procedure. Fish less than approximately six inches long and rainbow smelt 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. When this modification is used, it should be noted when reporting analytical results.

2. Some species are generally eaten by skinning the fish. The skin from these species is also relatively difficult to homogenize in the sample. Hence, for the following list of species, the fish is first skinned prior to filleting:

Brown bullhead	White catfish
Yellow bullhead	Channel catfish
Atlantic sturgeon	Lake sturgeon
Black bullhead	

3. American eel are analyzed by removing the head, skin, and viscera; filleting is not attempted.

4. Forage fish and young-of-year fish are analyzed whole. This category is considered to be less than 150mm (6 inches).

QAPP Appendix B – Laboratory SOPs

The QC criteria for CLP laboratories and the referenced SOPs for non-CLP laboratories are included in this appendix.

TestAmerica
THE LEADER IN ENVIRONMENTAL TESTING

Title: Acid Volatile Sulfides (AVS) and Simultaneously Extracted Metals (SEM) in Sediment

Approvals (Signature/Date):

5/12/2014	5/15/2014
Roseann Ruyechan Date Inorganics Department Manager	Steve Jackson Date Regional Safety Coordinator
5/15/2014	5/14/2014
Virginia Zusman Date Quality Assurance Manager	Deborah L. Lowe Date Laboratory Director

1.0 SCOPE AND APPLICATION

1.1. This method describes the procedure for the determination of acid volatile sulfides (AVS) and for metals that are solubilized during the acidification step (Simultaneously Extracted Metals, SEM). The conditions used have been reported to measure amorphous or moderately crystalline monosulfides. As a precipitant of toxic heavy metals, sulfide is important in controlling the bioavailability of metals in anoxic sediments. If the molar ratio of toxic metals measured by SEM to AVS exceeds one, the metals are potentially bioavailable. Because the relative amounts of AVS and SEM are important in the prediction of potential metal bioavailability, it is important to use the SEM procedure for sample preparation for metal analysis. This uses the same conditions for release of both sulfide and metal from the sediment and thus provides the most predictive means of assessing the amount of metal associated with the sulfide.

1.2. This method is applicable to solid matrices only, and is intended for analysis of sediment samples.

1.3. Method 9034 is used to quantify the concentration of sulfide and Method 6010B is used to quantify the concentration of the routine SEM metals (arsenic, cadmium, chromium, copper, lead, nickel, silver and zinc). If mercury is requested as a SEM, Method 7470A is used for quantification. Reporting limits are listed in Attachment 1.

1.4. On occasion clients may request slight modifications to this SOP. These modifications are handled as indicated PT-QA-M-001, Quality Assurance Manual.

2.0 SUMMARY OF METHOD

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. The amount of sulfide that is trapped is then determined titrimetrically following Method 9034. The SEM are metals liberated from the sediment during the acidification. These are determined following Method 6010B after filtration of the sample (plus 7470A if mercury is required).

3.0 DEFINITIONS

3.1. Acid Volatile Sulfides (AVS): Amorphous, moderately crystalline monosulfides, and other sulfides that form hydrogen sulfide under the conditions of this test.

3.2. Simultaneously Extracted Metals (SEM): Metals which form less soluble sulfides than do iron or manganese, and which are at least partially soluble under the conditions of this test. The routine SEMs are cadmium, copper, lead, nickel, and zinc. Mercury may also be determined on a project specific basis.

3.3. Refer to the glossary in the Laboratory Quality Manual (PT-QA-M-001), current version for additional definitions.

4.0 INTERFERENCES

4.1. Oxygen in the reagents and apparatus is the primary interference reported. Samples must be taken with minimum aeration to avoid volatilization of sulfide or reaction with oxygen, which oxidizes sulfide to sulfur compounds that are not detected. Use deoxygenated, deionized water and reagents.

4.2. Reduced sulfur compounds, such as sulfite and hydrosulfite, may decompose in acid and form sulfur dioxide. This gas may carry over to the zinc acetate solutions and subsequently react with iodine during the titration, thus causing a positive bias to the results.

4.3. The iodometric method suffers interference from reducing substances that react with iodine, including thiosulfate, sulfite, and various organic compounds.

4.4. The pH of the sample after the addition of the acid and during the purge process must be below 3.

5.0 SAFETY

Employees must abide by the policies and procedures in the Corporate Environmental Health and Safety Manual (CW-E-M-001) and this document. This procedure may involve hazardous material, operations and equipment. This SOP does not purport to address all of the safety problems associated with its use. It is the responsibility of the user of the method to follow appropriate safety, waste disposal and health practices under the assumption that all samples and reagents are potentially hazardous. Safety glasses, gloves, lab coats and closed-toe, nonabsorbent shoes are a minimum.

5.1. Specific Safety Concerns or Requirements

5.1.1. Hydrogen sulfide (H_2S) gas is generated by the addition of sulfuric acid. Inhalation of H_2S gas can cause headache, dizziness, nausea and unconsciousness and potentially death.

5.2. Primary Materials Used

The following is a list of the materials used in this method, which have a serious or significant hazard rating. **NOTE: This list does not include all materials used in the method. The table contains a summary of the primary hazards listed in the SDS for each of the materials listed in the table.** A complete list of materials used in the method can be found in

the reagents and materials section. Employees must review the information in the SDS for each material before using it for the first time or when there are major changes to the SDS.

Material (1)	Hazards	Exposure Limit (2)	Signs and symptoms of exposure
Hydrochloric Acid	Corrosive Poison	5 ppm-Ceiling	Inhalation of vapors can cause coughing, choking, inflammation of the nose, throat, and upper respiratory tract, and in severe cases, pulmonary edema, circulatory failure, and death. Can cause redness, pain, and severe skin burns. Vapors are irritating and may cause damage to the eyes. Contact may cause severe burns and permanent eye damage.
Iodine	Poison Corrosive Oxidizer	0.1 ppm-Ceiling	Vapors severely irritate and can burn the mucous membranes and respiratory tract. Liquid contact may cause blistering burns, irritation, and pain. Vapors may be severely irritating to the skin. Vapors are severely irritating and may cause damage to the eyes. Contact may cause severe burns and permanent eye damage.
Potassium Dichromate	Oxidizer Corrosive Carcinogen	0.1 Mg/M3 TWA as CrO3	Extremely destructive to tissues of the mucous membranes and upper respiratory tract. May cause ulceration and perforation of the nasal septum. Symptoms of redness, pain, and severe burn can occur. Dusts and strong solutions may cause severe irritation. Contact can cause blurred vision, redness, pain and severe tissue burns. May cause corneal injury or blindness.
Sodium Hydroxide	Corrosive	2 Mg/M3-Ceiling	Severe irritant. Effects from inhalation of dust or mist vary from mild irritation to serious damage of the upper respiratory tract, depending on severity of exposure. Symptoms may include sneezing, sore throat or runny nose. Contact with skin can cause irritation or severe burns and scarring with greater exposures. Causes irritation of eyes, and with greater exposures it can cause burns that may result in permanent impairment of vision, even blindness.
Sodium Sulfide	Corrosive	10 ppm-TWA 15 ppm-STEL	Will form Hydrogen Sulfide (HS) gas if combined with strong acids. Inhalation of HS gas may be fatal. Symptoms include painful conjunctivitis, headache, nausea, dizziness, coughing and, in extreme cases, pulmonary edema and possible death. Irritant. Contact with skin can produce serious caustic burns with painful inflammation and possible destruction of tissue. Inflammation, tearing and pain may be expected. Severe contact can cause destruction of tissue.
1 – Always add acid to water to prevent violent reactions.			
2 – Exposure limit refers to the OSHA regulatory exposure limit.			

5.3. Procedures shall be carried out in a manner that protects the health and safety of all TestAmerica associates.

5.4. Eye protection that protects against splash, laboratory coat, and appropriate gloves must be worn while samples, standards, solvents, and reagents are being handled. Disposable gloves that have been contaminated will be removed and discarded; other gloves will be cleaned immediately.

5.5. The health and safety hazards of many of the chemicals used in this procedure have not been fully defined. Additional health and safety information can be obtained from the Material Safety Data Sheets (SDS) maintained in the laboratory.

5.6. Exposure to chemicals must be maintained **as low as reasonably achievable**, therefore, unless they are known to be non-hazardous, all samples must be opened, transferred and prepared in a fume hood, or under other means of mechanical ventilation. Solvent and waste containers will be kept closed unless transfers are being made.

5.7. The preparation of standards and reagents will be conducted in a fume hood with the sash closed as far as the operation will permit.

5.8. All work must be stopped in the event of a known or potential compromise to the health and safety of a TestAmerica associate. The situation must be reported **immediately** to a laboratory supervisor or a TestAmerica Emergency Coordinator.

6.0 EQUIPMENT AND SUPPLIES

The following items are recommended for performing this procedure. Equivalent items should only be used when they result in an improvement in quality, efficiency, productivity, or cost. An item can be considered equivalent if with its use, the analytical and QA/QC requirements in this SOP can be met.

6.1. Instrumentation

6.1.1. Not Applicable.

6.2. Supplies

6.2.1. Boiling tube.

6.2.2. Inlet adapter.

6.2.3. Dropping funnel.

6.2.4. Gas inlet.

6.2.5. Impinged bubbler.

6.2.6. Fritted bubbler.

6.2.7. Bubbler vessels.

6.2.8. WestClips®

6.2.9. Gas line “T” connector.

6.2.10. Class A Volumetric flasks, pipettes, and burettes.

6.2.11. High purity nitrogen gas.

6.2.12. Regulator.

6.2.13. 100 mL and 300 mL graduated disposable flasks.

6.2.14. 100 mL disposable beaker

6.2.15. Hot plate stirrer.

6.2.16. 50mL burette.

6.2.17. Parafilm

6.2.18. Filtering apparatus and 0.45 μm filter membrane.

7.0 REAGENTS AND STANDARDS

7.1. Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee of Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

7.2. Reagent water (Super Q/DI Water). All references to water in this method refer to reagent water.

7.3. Zinc acetate for the scrubber. Zinc acetate solution (approximately 0.5M). Dissolve about 110g zinc acetate dihydrate in 200mL of reagent water. Add 1mL hydrochloric acid (concentrated), to prevent precipitation of zinc hydroxide. Dilute to 1L.

7.4. Acid to acidify the sample. 6 M Hydrochloric acid, 1:1 HCl:reagent water. Purge with nitrogen for at least 30 minutes prior to use.

7.5. UHP/zero grade nitrogen gas. Gas chromatographic grade with two-stage regulator.

7.6. Starch indicator. 0.5%. Purchased.

7.7. 0.0250N $Na_2S_2O_3.5H_2O$ Sodium Thiosulfate is prepared from certified ACS $Na_2S_2O_3.5H_2O$ by weighing 24.82g, diluting to 4L in reagent water and preserving with 36 mL of 1N NaOH. (NOTE: Weights/Volumes can be adjusted as long as proportionality is maintained.)

7.8. 0.025N Iodine. Purchased.

7.9. 1000ppm Sodium Sulfide LCS/MS spiking solution is prepared by adding approximately 1.88g $Na_2S•9H_2O$ to 250mL reagent water or an equivalent spike that has been titrometrically determined due to loss of sodium sulfide due to atmospheric oxygen and/or water vapor. (NOTE: Weights/Volumes can be adjusted as long as proportionality is maintained.)

7.10. 1000ppm Sodium Sulfide ICV (second source) spiking solution is prepared by adding approximately 1.88g Na2S•9H2O to 250mL reagent water or an equivalent spike that has been titrometrically determined due to loss of sodium sulfide due to atmospheric oxygen and/or water vapor. (NOTE: Weights/Volumes can be adjusted as long as proportionality is maintained.)

7.11. ICP Stock Spiking Solution, purchased standard. See PT-MT-001 for the concentration of elements in the Stock standard.

8.0 SAMPLE COLLECTION, PRESERVATION, SHIPMENT AND STORAGE

Sample container, preservation techniques and holding times may vary and are dependent on sample matrix, method of choice, regulatory compliance, and/or specific contract or client requests. Listed below are the holding times and the references that include preservation requirements.

Matrix	Sample Container	Minimum Sample Size	Preservation	Holding Time	Reference
AVS-Sediment	Glass	4 oz jar	Cool ≤ 6.0°C	14 days from collection to analysis	40 CFR Part 136
SEM -Metals	Glass	4 oz jar	Cool ≤ 6.0°C	180 days from collection to analysis	40 CFR Part 136
SEM - Mercury	Glass	4 oz jar	Cool ≤ 6.0°C	28 days from collection to analysis	40 CFR Part 136

8.1. The acidification of the sample (H_2S generation) and sulfide determination must be

performed within 14 days from the date of collection. The routine SEMs are stable up to six months after sample collection (28 days for mercury, if required).

8.2. If after distillation, the AVS distillate cannot be immediately titrated it may be stored at ≤ 6.0°C for up to 24 hours before final titration provided the 14 day holding time is not exceeded.

9.0 QUALITY CONTROL

9.1. The following quality control samples are prepared with each batch of AVS samples.

Quality Controls	Frequency	Control Limit
ICV (second source standard)	Daily prior to sample analysis	85 -115%
ICB/Prep Blank	Daily prior to sample analysis	< RL
Method Blank (MB)	1 per preparation batch[1]	< 2 times RL
Laboratory Control Sample (LCS)	1 per preparation batch[1]	85-115 %
CCV	After every 10 samples	85-115 %
CCB	After every 10 samples	< RL
Matrix Spike (MS)[3]/MSD	1 per preparation batch[1]	75-125[2]% and 20% RPD

[1]A batch is limited to 20 samples.
[2]Statistical control limits will be developed when there is sufficient data and updated as per SOP PT-QA-021.
[3]The MS/MSD is randomly selected, unless specifically requested by a client.

9.2. Sample QC

9.2.1. A separate sulfide (AVS) LCS and metals (SEM) LCS is performed. Teflon chips are used in the extraction procedure to simulate a solid matrix. See SOP PT-MT-001 for the 6010B LCS criteria or SOP PT-MT-005 for the 7470A LCS criteria.

9.2.2. A separate sulfide (AVS) MS/MSD and metals (SEM) MS/MSD is performed. See SOP PT-MT-001 for the 6010B MS/MSD criteria or SOP PT-MT-005 for the 7470A MS/MSD criteria.

9.2.2.1. The percent recovery for matrix spike and matrix spike duplicate should be ± 25 percent. If this criterion is not met, evaluate method process. If no errors are found, document in a Non-Conformance Memo (NCM).

9.2.2.2. The relative percent difference (RPD) between the MS and MSD must be within ± 20 percent. If this criterion is not met, then repeat the analysis once. The results with the better RPD will be

reported. If the results for the reanalysis are the same as the original analysis, then report the original analysis.

9.2.3. The prep blank (or method blank) can be used as the ICB if it meets the ICB acceptance criteria. The processing of a method blank will assure non-contamination of the reagents. Teflon chips are used in the extraction procedure to simulate a solid matrix. The ICB result must be less than the Reporting Limit. The method blank result must be less than two times the Reporting Limit, otherwise all samples must be reprepared and reanalyzed. If this is not possible due to limited sample quantity (or there is no sample left) the corresponding samples will be flagged and the PM will be notified. If repreparation and reanalysis happen to be outside holding time, then approval from the client must be obtained before any reanalysis is performed. See SOP PT-MT-001 for the 6010B Method Blank criteria or SOP PT-MT-005 for the 7470A Method Blank criteria.

9.3. Instrument QC

9.3.1. The ICV is an undistilled standard prepared by adding 1 mL of a 1000 ppm (or standardized concentration) sodium sulfide standard (different source than the standard used for the LCS and MS/MSD) to 50 mL of DI water (20 ppm concentration).

9.3.2. The ICB is an undistilled blank consisting of 50 mL of reagent water.

9.3.3. A sulfide run will consist of the following sequence: ICV, ICB, and up to 10 samples followed by a CCV and a CCB. **See the appropriate metals SOP for the SEM analyses.**

9.3.4. This can be followed by up to 10 more samples, followed by a CCV and CCB.

9.3.5. Repeat 9.3.1 and 9.3.2 sequence for additional samples.

9.3.6. The following QC requirements must be met for the sulfide (AVS) analyses:

9.3.6.1. The ICV must be within ± 15 percent. If this criterion is not met, then restandardize and reanalyze the samples. The LCS can be used as an ICV if it meets the ICV acceptance criteria of 85 to 115 percent. If the LCS is not used as the ICV, then the LCS must meet a 75 to 125 percent recovery criterion.

9.3.6.2. The CCV must be within ± 15 percent. If this criterion is not met, then reanalyze the samples with a valid CCV. If the analysis sequence shows ICV, ICB, and 10 samples followed by CCV,

CCB, and this CCV fails, then all those 10 samples must be reanalyzed. If with the above sequence 10 additional samples are analyzed following a CCV and a CCB and this second CCV fails, then all the samples up to the last acceptable CCV must be reanalyzed. The CCB criteria are the same as ICB.

9.4. All sample preparation and analysis information will be documented directly into TALS LIMS.

10.0 PROCEDURE

10.1. Sample Preparation

10.1.1. Place the boiling tube containing approximately 10 grams of sample (record to the nearest 0.1 grams) and 50 mL of reagent water in the heater block (used as a holder only) and assemble the acid soluble sulfide distillation apparatus as shown in Figure 1. The sample can be weighed on a 2" x 2" piece of Parafilm and placed into the boiling tube.

10.1.2. Spike the sulfide (AVS) LCS, MS, and MSD with 1 mL of the 1000 ppm sodium sulfide solution (7.11) which is equivalent to 100 mg/Kg in a 10 gram sample. Spike the metals (SEM) LCS, MS, and MSD with 2.5 mLs of the metals ICP MS solution. If mercury is required, 0.25 mLs of the intermediate mercury spike will need to be added to the LCS, MS, and MSD.

10.1.3. Place 2.0mL of 0.5M zinc acetate solution and 20.0mL of deionized water in each of two bubbler vessels. Place an impinged bubbler in the first (front) and second (back) vessel, and seal them with size 24/40 WestClips®. The sealed vessels and impingers function as the gas scrubbers. Connect the first scrubber to the inlet adapter and place the second bubbler vessel in the bubbler vessel rack. Connect the two impingers in series using Tygon® tubing.

10.1.4. Close stopcock of dropping funnel. Place 20 mL of the nitrogen purged 6 M hydrochloric acid in the dropping funnel.

10.1.5. Connect a high-purity (GC grade) nitrogen gas source to the main inlet of the gas manifold of the aluminum heater block as specified in the Heater Block Operation Manual. Use a two-stage gas tank regulator and set the pressure into the gas manifold to 20psi.

10.1.6. Connect a black gas line from each gas manifold valve to a "T" connector and a tygon gas line from the "T" to each of the two gas inlets of the apparatus. One at the top of the dropping funnel and one at the inlet adapter as shown in Figure 1.

10.1.7. Purge assembled apparatus with high-purity nitrogen for 10 minutes to remove atmospheric oxygen from the apparatus and contained solutions. During purge, adjust nitrogen flow such that 2-3 bubbles per second exit the base of the inlet adapter.

10.1.8. Open stopcock of dropping funnel and allow all of the 6M hydrochloric acid to drip into the boiling tube. Once dropping funnel is empty, close the stopcock to ensure sample is not lost into the funnel.

10.1.9. Purge the sample for 1 hour at room temperature. After the 1 hour purging period, remove the bubbler vessels. Turn off the nitrogen flow. Carefully combine the gas scrubber solutions in a 100 mL graduated disposable flask. Do not shake or mix solutions to avoid loss of sulfide. Bring up to 50 mL with reagent water. Determine the concentration of acid volatile sulfide in the zinc acetate gas scrubber solutions by using the Titrimetric-iodine method (9034)—proceed to Section 10.3.

10.1.10. After the generation of sulfide has been completed, the sediment suspension remaining in the boiling tube is filtered through a 0.45 μm membrane filter. The pH of the solution is determined using narrow range pH strips to verify that the pH is less than 3. If the pH is not less than 3, the group supervisor and QA Manager should be consulted. Document all actions in a Nonconformance Memo (NCM). The solution is brought up to a final volume of 250 mL in a 300 mL graduated disposable flask. This solution is analyzed directly by ICP for the routine SEMs (see SOP PT-MT-001). If mercury is required, an aliquot of this solution is prepared following Method 7470A and analyzed by CVAA (see SOP PT-MT-005).

10.2. Calibration

NOTE: All periodic standardizations of titrants can be found in the Wet Chemistry standardization logbook. Daily standardizations are found on the TALS worksheet.

10.2.1. Stock sulfide standard is titrated daily before each distillation of sample sets. The stock standard must be reprepared every week.

10.2.2. Sodium thiosulfate (0.0250N) standardization—performed daily.

10.2.2.1. Use 0.0250 N Biiodate titrant: dissolve 0.8124g potassium biiodate (dried 2 hours) in Super-Q water and dilute to 1 L.

10.2.2.2. Place 2g KI in 250mL beaker and add 100mL Super-Q water and stir. Add 10mL 1:4 H_2SO_4 and 10mL biiodate.

10.2.2.3. Place in the dark for 5 minutes. Dilute to 150mL and add starch indicator (Section 7.8)

10.2.2.4. Titrate with $Na_2S_2O_3$ (7.9) to clear endpoint. Repeat procedure two additional times. Determine the normality of the $Na_2S_2O_3$ as follows:

$$N\ Na_2S_2O_3 = \frac{10\ mL\ biiodate \times 0.025\ N\ biiodate}{mL\ Na_2S_2O_3\ titrant}$$

10.2.3. Iodine standardization: performed daily

10.2.3.1. Place 20mL .0250N iodine in Erlenmeyer flask. Add 2mL 6 N HCl.

10.2.3.2. Titrate with $Na_2\ S_2O_3$ (7.9) to a pale yellow color.

10.2.3.3. Add starch indicator (7.8) and titrate with $Na_2S_2O_3$ (7.9) to clear endpoint. Determine the normality of the iodine (I) as follows:

$$N\ I = \frac{N Na_2S_2O_3 \times mL\ Na_2S_2O_3\ titrant}{mL\ I\ solution}$$

10.3. Sample Analysis

10.3.1. Pipette a known amount of standardized 0.025N iodine solution into the 100mL disposable sample beaker containing 50 mL of sample, adding an amount in excess of that needed to oxidize the sulfide.

10.3.2. Add 2mL of 6N HCl to the iodine.

10.3.3. If at any point the amber/orange color of the iodine disappears or fades to yellow, more 0.025N iodine must be added. This additional amount must be added to the amount from Section 10.3.1 for calculations. Record the total volume of standardized 0.025N iodine solution used.

10.3.4. Add enough starch indicator (approximately 1 mL) for the solution to turn a dark blue color.

10.3.5. Titrate the solution in the flask with standard 0.025N sodium thiosulfate solution until the dark blue color disappears. Record the volume of titrant used.

10.3.6. For metals, the solution is analyzed directly by ICP for the routine SEMs (see SOP PT-MT-001). If mercury is required, an aliquot of this solution is prepared following Method 7470A and analyzed by CVAA (see SOP PT-MT-005).

11.0 CALCULATIONS / DATA REDUCTION

11.1. One mL of 0.0250 N standard iodine solution reacts with 0.4mg sulfide present in titration vessel.

11.2. $$\text{AVS mg/Kg-dry} = \frac{[(A \times B) - (C \times D)] \times 16000}{E \times F}$$

A = mL of iodine solution
B = N of iodine solution
C = mL of $Na_2S_2O_3$ solution
D = N of $Na_2S_2O_3$ solution
E = weight of sample (grams or mls)
F = Percent solids as decimal fraction (i.e., 50% solid is 0.50)

11.3. To convert the AVS concentration from mg/Kg-dry to μmoles/gram-dry, divide by 32.066 (molecular weight of sulfur).

11.4. Enter the completed data work sheet into computer program, sulfide analysis worksheet, for final results.

11.5. For each SEM, first determine concentration in mg/Kg-dry as follows:

$$\text{SEM mg/Kg-dry} = \frac{A \times B}{C \times D}$$

A = conc. of metal in solution as determined by 6010B or 7470A (mg/L)
B = final volume of solution in liters—typically 0.25 liters.
C = weight of sample in Kg.
D = Percent solids as decimal fraction (i.e., 50% solid is 0.50)

11.6. To convert the concentration of each SEM from mg/Kg-dry to μmoles/gram-dry, divide by the molecular weight of that metal (cadmium = 112.411; copper = 63.546; lead = 207.2; mercury = 200.59; nickel = 58.69; and zinc = 65.39).

11.7. Calculate the Total SEM molar concentration of the sample by summing each of the individual SEM concentrations in units of μmoles/gram-dry. If any one of the SEMs is not detected (ND), it is considered a zero (0) in the summation.

11.8. Calculate the molar ratio of SEM over AVS as follows:

SEM/AVS = A/B

A = Total SEM molar concentration (μmoles/gram-dry).
B = AVS molar concentration (μmoles/gram-dry).

Note: If AVS is not detected (ND), the molar ratio cannot be determined.

11.9. Matrix Spike percent recovery:

$$Theoretical\ Spike\ Conc. = \frac{\begin{pmatrix} Spike \\ Conc. \end{pmatrix} \times \begin{pmatrix} Vol.\ of \\ Spike\ Added \end{pmatrix}}{Final\ Vol.\ Spiked}$$

$$\%\ \text{Recovery} = \frac{\begin{pmatrix} Final \\ Spike + Sample \end{pmatrix} - Sample\ \text{Result}\begin{pmatrix} Vol.\ Sample \\ Spiked \\ \hline Final\ Vol. \\ Spiked \end{pmatrix}}{Theoretical\ Spike\ Conc.} \times 100$$

12.0 METHOD PERFORMANCE

12.1. The supervisor has responsibility to ensure that an analyst who performs this procedure is properly trained in its use and has the required experience. Performance is monitored through internal QC and outside performance evaluation samples. Please refer to the QA Manual for additional information concerning Precision and Accuracy.

12.2. Demonstration of Capabilities – Prior to the analysis of samples, a Demonstration of Capabilities (DOC) as described in the QA Manual, must be performed initially, annually and any time a significant change is made to the analytical system.

12.3. Method Detection Limit Study – A Method Detection Limit (MDL) study, as described in the QA Manual, must be performed initially, annually and any time a significant change is made to the analytical system.

13.0 POLLUTION CONTROL

13.1. It is TestAmerica's policy to evaluate each method and look for opportunities to minimize waste generated (i.e., examine recycling options, ordering chemicals based on quantity needed, preparation of reagents based on anticipated usage and reagent stability). Employees must abide by the policies in Section 13 of the Corporate Environmental Health and Safety Manual (CW-E-M-001) for "Waste Management and Pollution Prevention."

13.2. This method does not contain any specific modifications that serve to minimize or prevent pollution.

14.0 WASTE MANAGEMENT

14.1. Waste management practices are conducted consistent with all applicable rules and regulations. Excess reagents, samples and method process wastes are disposed of in an accepted manner. Waste description rules and land disposal restrictions are followed. Waste disposal procedures are incorporated by reference to SOP PT-HS-001. The following waste streams are produced when this method is carried out.

14.1.1. Acidic waste generated by sample titration. This waste is collected in a waste container identified as "Acid Waste", Waste #33. This waste is neutralized to a final pH between 6 and 9 and discharged down into a lab sink.

14.1.2. Unused sample distillate. This waste is collected in a waste container identified as "Acid Waste", Waste #33. This waste is neutralized to a final pH between 6 and 9 and discharged down into a lab sink.

14.2. Waste generated in the procedure will be segregated, and disposed according to the facility hazardous waste procedures. The Environmental Health and Safety Coordinator should be contracted if additional information is required.

15.0 REFERENCES

15.1. Test Methods for Evaluating Solid Waste, Physical/Chemical Methods, 3rd ed.; U.S. EPA. Office of Solid Waste and Emergency Response. U.S. Government Printing Office: Washington, DC, 1997; SW-846.

15.2. Allen, H.E. and F. Gongmin et al. 1991. Determination of Acid Volatile Sulfide and Simultaneously Extractable Metals in Sediment, April 1991 (Draft Analytical Method for the Determination of Acid Volatile Sulfide in Sediment, U.S. EPA Office of Water and Office of Science and Technology, Health and Ecological Criteria Division, Washington, D.C., August 1991. EPA-182-R-91-100.

15.3. SOP PT-MT-001, Inductively Coupled Plasma-Atomic Emission Spectroscopy, Spectrometric Method for Trace Element Analyses, SW-846 Method 6010B, 6010C and EPA Method 200.7, current revision.

15.4. SOP PT-MT-005, Preparation and Analysis of Mercury in Aqueous Samples by Cold Vapor Atomic Absorption, SW-846 7470A and MCAWW 245.1, current revision.

15.5. SOP PT-WC-010, Total Sulfide as Acid Soluble Sulfide, Method 9030B/9034, SM 20th Ed. 4500S-$^{2-}$F, current revision.

15.6. SOP PT-QA-007, Detection Limits, current revision.

15.7. SOP PT-QA-003, Glassware Clean-up for Organic/Inorganic Procedures, current version.

15.8. SOP PT-QA-006, Procurement of Standards and Materials; Labeling and Traceability, current version.

15.9. SOP PT-QA-011, Data Recording Requirements, current version.

15.10. SOP PT-QA-012, Selection and Calibration of Balances and Weights, current version.

15.11. SOP PT-QA-016, Nonconformance & Corrective Action System, current version.

15.12. SOP PT-QA-021, Quality Assurance Program, current version.

15.13. SOP PT-QA-022, Equipment Maintenance, current version.

15.14. SOP PT-SR-001, Sample Receiving & Login, current version.

15.15. SOP PT-QA-031, Internal Chain of Custody, current version.

15.16. PT-QA-M-001, Pittsburgh Quality Assurance Manual, current version.

16.0 METHOD MODIFICATIONS

16.1. Not applicable.

17.0 ATTACHMENTS

17.1. Figure 1 – Acid Volatile Sulfide generation apparatus.

17.2. Attachment 1 – SEM and AVS Reporting Limits. MDLs listed in the two attachments for metals and sulfide are subject to change.

18.0 REVISION HISTORY

18.1. Revision 1, 5/16/2008.

18.2. Revision 2, 5/27/2010

18.3. Revision 3, 11/10/2010

18.4. Revision 4, 11/18/2011

18.5. Revision 5, 5/15/2014

SOP section	Change from	Change to	Reason
Cover	Steve Jackson – Health & Safety Manager/ Coordinator QAM – Nasreen DeRubeis Technical Specialist – Mike Wesoloski	Regional Safety Coordinator QAM –Virginia Zusman Roseann Ruyechan – Inorganics Department Manager	Change in personnel
Entire SOP	Updated	PT-LQAM to PT-QA-M-001	SOP numbering change
1.3	Added	SOP Review Checklist text on method modifications	SOP Review Format
3.3 through 3.8	Removed	SOP Review Checklist text on definitions contained in the QA Manual	SOP Review Format
3.3 and 3.4	Moved	9.3.1 – ICV and 9.3.2 - ICB	Correction
5.2	Removed	Radiation Safety Manual	Does not pertain to this facility
5.4	Removed	“ANSI Z87.1” and added “protects against splash”	Correction
5.5	Updated	MSDS to SDS	Correction
6	Added	Equivalency statements from SOP Review Checklist	SOP Review Sheet format
7.7	Updated	Included directions on how the sodium thiosulfate reagent is prepared by the laboratory	Clarification
7.9	Updated	Section 7.9 for the 1000 ppm sodium sulfide spiking solution to be consistent with SOP PT-WC-	Correction

		010	
7.10	Added	Section 7.10 for the ICP Stock spiking solution	Correction
8	Added	Requirement text for sample containers, preservation and holding times from SOP Review Checklist and added this information into a Table format	SOP Review Sheet format
8.2	Updated	Changed 6 to 6.0 and 7 day holding time to 14 days.	Correction
9.1	Added	Added the AVS QC information into a Table format	Clarification
9.2.1 and 9.2.3	Added	Noted that Teflon chips are used to simulate a solid matrix	Correction
9.2.1, 9.2.2 and 9.2.3	Added	Referenced the 6010B and 7470A SOP's for the LCS, MS/MSD and MB requirements	Clarification
9.3	Removed – section not necessary	In the NOTE under section 10.2 replaced bench with TALS in the second sentence	Correction
9.3.3	Updated	Section references to 9.3.1 and 9.3.2	Correction
9.3.4.1	Updated	Changed recalibrate to restandardize in the second sentence	Correction
9.4	Updated	Noted all prep and analysis information is recorded directly into TALS LIMS	Correction
10.1.2	Added	"0.25 mLs of the intermediate" mercury spike to the last sentence	Correction
10.2	Updated	In the NOTE under section 10.2 replaced bench with TALS in the second sentence.	Correction
12.1	Added	Supervisor responsibility text from SOP Checklist; removed old text	SOP Review Sheet format
12.2	Added	DOC text from SOP Checklist; removed old text	SOP Review Sheet format
12.3	Added	MDL Text from SOP Checklist; removed old text	SOP Review Sheet format
15.14	Updated	PT-QA-027 to PT-SR-001 Sample Receiving & Login SOP	Correction
15.15	Added	PT-QA-031 Internal Chain of Custody SOP	Correction

Attachment 1 – Reporting Limits

TAL Reference Data Summary

Structured Analysis Code: A-**-OS-8G-03
Target Analyte List: All Analytes

Matrix: SOLID
Extraction: None specified.
Method: Simultaneously Extractable Metals in Sediment
QC Program: MARINE\SEDIMENT
Location: TestAmerica Pittsburgh

Analyte List		Detection Limits					Check List 3008							Spike List 3009						
Syn	Compound	RL	Units	MDL	Units	Run Date	T	A	Amt	Units	LCL	UCL	RPD	T	A	Amt	Units	LCL	UCL	RPD
140	Arsenic	0.003337	umoles/gn	0.00038(	umoles/gn	20061013	C	Y	0.6673	umoles/g	80	120	20	C	Y	0.6673	umoles/g	75	125	20
411	Cadmium	0.001112	umoles/gn	0.00003(	umoles/gn	20061013	C	Y	0.0111	umoles/g	80	120	20	C	Y	0.0111	umoles/g	75	125	20
2952	Chromium	0.002404	umoles/gn	0.00021(	umoles/gn	20061013	C	Y	0.0961	umoles/g	80	120	20	C	Y	0.0961	umoles/g	75	125	20
643	Copper	0.009835	umoles/gn	0.00088:	umoles/gn	20061013	C	Y	0.0983	umoles/g	80	120	20	C	Y	0.0983	umoles/g	75	125	20
1605	Lead	0.0007239	umoles/gn	0.00023(	umoles/gn	20061013	C	Y	0.0603	umoles/g	80	120	20	C	Y	0.0603	umoles/g	75	125	20
1701	Mercury	0.0000623	umoles/gn	0.000006	umoles/gn	20061013	C	Y	0.0001	umoles/g	80	120	20	C	Y	0.0001	umoles/g	80	120	20
1956	Nickel	0.01704	umoles/gn	0.00049(	umoles/gn	20061013	C	Y	0.2129	umoles/g	80	120	20	C	Y	0.2129	umoles/g	75	125	20
2285	Silver	0.001159	umoles/gn	0.00013!	umoles/gn	20061013	C	Y	0.0115	umoles/g	80	120	20	C	Y	0.0115	umoles/g	75	125	20
2649	Zinc	0.03823	umoles/gn	0.00282(	umoles/gn	20061013	C	Y	0.1911	umoles/g	80	120	20	C	Y	0.1911	umoles/g	75	125	20

Attachment 1 – Cont.

TAL Reference Data Summary

Structured Analysis Code: A-**-P7-8G-03
Target Analyte List: All Analytes

Matrix: SOLID
Extraction: None specified.
Method: Acid Volatile Sulfide in Sediment (AVS)
QC Program: MARINE\SEDIMENT
Location: TestAmerica Pittsburgh

Analyte List			Detection Limits				Check List 3000							Spike List 3001						
Syn	Compound	RL	Units	MDL	Units	Run Date	T	A	Amt	Units	LCL	UCL	RPD	T	A	Amt	Units	LCL	UCL	RPD
3735	Acid Volatile Sulfide	0.499	umoles/gn	0.155	umoles/gn	20050101	C	Y			85	115	20	C	Y			75	125	25

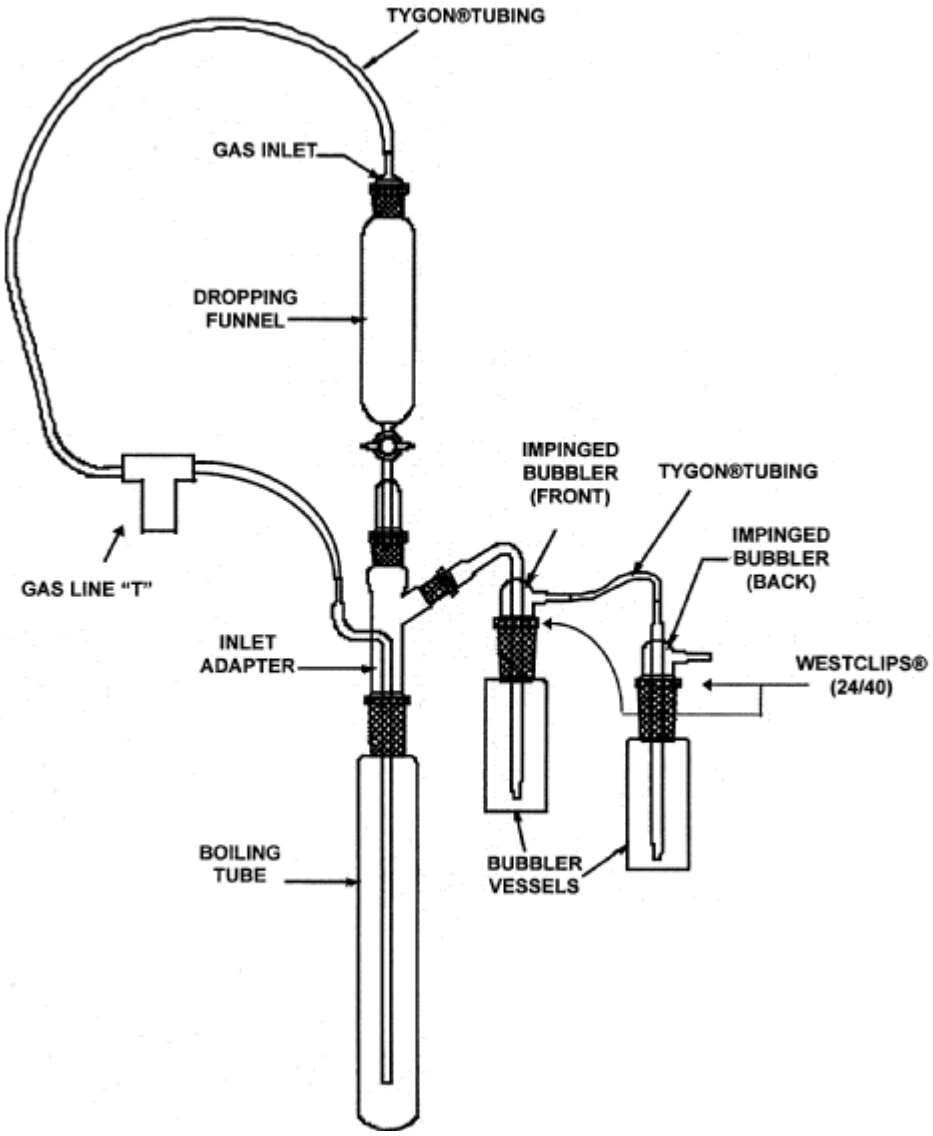


Figure 1 – Acid Volatile Sulfide generation apparatus.

STANDARD OPERATING PROCEDURE FOR 28-DAY AND 42-DAY LIFE-CYCLE WHOLE SEDIMENT TOXICITY TESTS WITH *Hyalella azteca*

SED 7003

Method Reference: ASTM 1706-95b and 1391-94 and EPA/600/R-99/064

January 2012

Great Lakes Environmental Center, Inc.
(GLEC)

Mailee Garton — 1/6/12
Mailee Garton
Technical Author
Date

Michelle VanDenBrand — 1/9/12
Michelle VanDenBrand
User Reviewer
Date

Dennis McCauley — 1/6/12
Dennis McCauley
President/ Principal Research Scientist
Date

Jennifer Hansen — 1/6/12
Jennifer Hansen
GLEC Quality Assurance Officer
Date

TABLE OF CONTENTS

I. SCOPE AND APPLICATION

1.1 This SOP describes the methodology for conducting 42 day whole sediment toxicity tests with *Hyalella azteca*. Test duration may be shortened to only evaluate survival and growth endpoints at 28 days.

1.2 *H. azteca* (a freshwater amphipod) is a commonly used test organism in freshwater and estuarine whole sediment toxicity tests. The 42 day protocol is a chronic or long-term bioassay that evaluates the effect of contaminated sediment on organism survival, growth, and reproduction. In the 42 day protocol, the test organisms are removed from the sediment exposure chambers into freshwater and fed. Reproduction (if any) is monitored through test termination at 42 days. In a 28 day test, the test organisms are removed and weighed to determine growth.

1.3 This SOP must be used in combination with hands on training directed by the toxicology laboratory coordinator or other qualified personnel. Documentation of completed training will be kept by administrative staff and the toxicology laboratory coordinator.

II. SUMMARY OF METHOD

2.1 During this test, organisms are continuously exposed to sediment samples for 42 days with intermittent (minimum of twice daily) renewal of overlying water. *Hyalella* survival and growth are recorded at 28 days, and survival, growth and reproduction, are recorded at 42 days (test termination). The survival, growth and reproduction data are used to determine if the whole sediment is toxic (measured by reduced survival, growth or reproduction) relative to a suitable reference control or laboratory control sediment.

2.2 This SOP is based on *Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates*, EPA/600/R-99/064; ASTM 1706-95b, *Standard Test Methods for Measuring the Toxicity of Sediment associated Contaminants with Fresh Water Invertebrates;* and ASTM 1391-94, *Standard guide for Collection, Storage, Characterization, and Manipulation of Sediments for Toxicological Testing.*

III. DEFINITIONS

3.1 Contaminated sediment – Sediment containing chemical substances at elevated concentrations that pose a known or suspected threat to aquatic life or human health.

3.2 Control sediment – An uncontaminated sediment (i.e., clean field collected or a formulated sediment that is prepared in the laboratory) that is essentially free of contaminants and is used routinely to assess the acceptability of a test and health of the test organisms.

3.3 Dechlorinated tap water - Municipal tap water is dechlorinated at GLEC using carbon-bed tanks. The dechlorinated water is checked weekly in the laboratory, and must contain less than or equal to .02 ppm of chlorine.

3.4 Deionized (DI) water – Water that has had its mineral ions removed. It is produced by purifying tap water by reverse osmosis (RO) followed by passing it through carbon and de-ionization cartridges. It is a physical process using ion exchange resins which bind to and filter out the mineral salts from water. (GLEC's DI Water meets Type II Reagent Water specifications.) Deionized water system filters are changed when the resistivity reading is lower than 15 Megohms-cm (MΩ-cm).

3.5 Formulated sediment – Mixtures of materials (e.g., clay, sand, and organic material) used to mimic the physical components of natural sediment. Formulated sediment is prepared in the laboratory and may be used as a substitute for the field collected reference sediment or the laboratory control sediment.

3.6 Sediment – Particulate soil material that usually lies below the water surface.

3.7 Whole sediment – Field collected sediment and associated pore (interstitial) water that has had minimal manipulation.

3.8 Overlying water – water that is placed over sediment in a test chamber during a test. Usually dechlorinated water, but depending on the study plan may be pristine surface water, or water provided by the client.

3.9 Pristine surface water – Field collected laboratory filtered culture water. Pressure filtered using a 1.2 µm nylon filter and nitrogen gas.

3.10 Reconstituted water - Synthetic dilution water, prepared with deionized water and reagent grade chemicals or mineral water, to achieve measurable water quality values of pH, hardness, and alkalinity.

3.11 Reference sediment – An uncontaminated whole sediment collected near an area of concern and used to assess sediment conditions exclusive of the contaminants of interest.

IV. INTERFERENCES AND CAUTIONS

4.1 Interferences are characteristics of a sediment or sediment test system other than those related to sediment-associated chemicals of concern, which can potentially affect toxicity test results and test organism survival, growth, or reproduction.

4.2 Interferences can potentially confound interpretation of test results in two ways: (1) false-positive response, i.e., toxicity is observed in the test when contamination is not present at concentrations known to elicit a response, or there is more toxicity than expected; and (2) false-negative response, i.e., no toxicity or bioaccumulation is observed when contaminants are present at concentrations known to elicit a response, or there is less toxicity or bioaccumulation than expected.

4.3 Interfering factors that can cause false-negative or false-positive responses:

4.3.1 Physical and chemical characteristics of sediment affecting test organisms independent of chemical concentration (i.e., non-contaminant factors), such as grain size, acid volatile sulfides, total organic carbon, and redox potential.

4.3.2 Changes in chemical bioavailability as a function of sediment manipulation or storage, such as aeration, volatilization, reduction, and oxidation.

4.3.3 The presence of predatory indigenous organisms.

4.4 Equipment and supplies that contact sediment and overlying water should be chosen to minimize sorption of test materials from the water or sediment and must not add toxicity. Glass, type 316 stainless steel, nylon, high-density polyethylene, polypropylene, polycarbonate, and fluorocarbon plastics should be used whenever possible to minimize leaching, dissolution, and sorption.

V. HEALTH AND SAFETY

5.1 Each whole sediment sample should be regarded as a potential health hazard and exposure should be as low as reasonably achievable.

5.2 Technicians must wear appropriate protective clothing, at a minimum a lab coat, safety glasses and disposable (latex or nitrile) gloves, while working near the samples during the testing period while disposing of test materials, and when cleaning glassware or equipment.

5.3 Contaminated sediment is often very aromatic. The preparation of sediment for use in toxicity tests should always be conducted in a well ventilated work area or under a fume hood.

VI. EQUIPMENT AND SUPPLIES

6.1 Analytical balance capable of accurately weighing to 0.01 mg.

6.2 Aluminum weigh boats.

6.3 Carbouy.

6.4 Desiccator.

6.5 Droppers, and glass tubing with fire polished edges, 4- to 6-mm ID—for transferring test organisms.

6.6 Drying oven.

6.7 Environmental chamber or water bath; temperature controlled.

6.8 Forceps.

6.9 Juvenile test organism holding beakers.

6.10 Lab hood or sample handling ventilation system.

6.11 Larval test organism rearing chambers.

6.12 Light box.

6.13 Log Books.

6.13.1 Client sediment log book.

6.13.2 Test materials or Sample Log Book .

6.13.3 YTC Log Book.

6.14 Meters

6.14.1 Dissolved oxygen (DO), pH/selective ion, ammonia, and specific conductivity meters and probes for routine chemical measurements.

6.14.2 Light meter.

6.15 Pipet bulbs and pipets (serological pipets: 1 to 10 mL, graduated).

6.16 Shallow glass or plastic pans.

6.17 Test chambers; 470 mL glass (pint) jar with nylon screened drain hole measured at 275 mLs and an equal number of 300 mL high form lipless beakers with a screened drain hole at 175 mL with a piece of Nytex® screen for the reproduction phase of the toxicity test (42 day tests only).

6.18 Thermometer(s) –

6.18.1 handheld digital.

6.18.2 data temperature logger.

6.18.3 NIST certified bulb thermometer.

6.19 Reference weights, Class 4: for documenting the performance of the analytical balance(s). The balance(s) should be checked with reference weights that are at the upper and lower ends of the range of the weightings made when the balance is used.

6.20 Sieves: #45 and #60 for collecting test organisms.

6.21 Stainless steel bowls and spoons.

6.22 Volumetric flasks and graduated cylinders – Class A, borosilicate glass or nontoxic plastic labware10 to 1000 mL for making test solutions.

6.23 Wash bottles – for rinsing small glassware, instrument electrodes and probes with DI water.

6.24 YTC-Yeast Trout Chow, and Cerophyl, 1800 mg/L.

VII. REAGENTS AND STANDARDS

7.1 Gases – Not Applicable. No gases are used in this procedure.

7.2 Reagent Water –

7.2.1 DI water.

7.2.2 Dechlorinated laboratory water.

7.2.3 Reconstituted water.

7.2.4 Other – as needed as per study plan for overlying water that is other than dechlorinated water.

7.3 Reagents – Not Applicable. No reagents are used in this procedure.

7.4 Standard Solutions – Sugar-formalin; prepare by mixing 120 g of sucrose with a 80 mL formalin solution and diluting the mixture to one liter with DI water. This solution is used to preserve test organisms.

7.5 Biological Specimens – *Hyalella azteca.*

VIII. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

See the following GLEC SOP's for further information: TOX 0001, *Standard Operating Procedure for Storage and Handling of Test Materials;* SED 7002, *Standard Operating Procedure for Whole Sediment Sample Collection and Shipment for Toxicological Tests*; and TOX 0002, *Standard Operating Procedure for Effluent and Receiving Water Collection and Shipment*.

IX. QUALITY CONTROL

9.1 Data sheets and laboratory log books will be maintained as indicated in this SOP. These are reviewed by the toxicology technical supervisor.

9.2 Clean glassware and associated equipment in the prescribed manner (GLEC SOP #TOX 1020 *Manual Cleaning of Glassware and Equipment Used in Toxicity Tests).*

9.3 *Hyalella azteca* – Minimum mean control survival must be 80% or greater on days 28 and 42.

9.4 *Hyalella azteca* – Minimum mean growth (measured as dry weight) at day 42 recommended to be greater than 0.15 mg/organism.

9.5 *Hyalella azteca* – Reproduction from day 28 to day 42 is recommended to be > 2 young/female.

9.6 Archive 20-80 test organisms for initial weight or length determination.

9.7 Reference toxicant testing is conducted (along with each toxicity test) using sodium chloride as the toxicant with each batch of organisms used to initiate a test. Control charts generated from the reference toxicity testing results are provided with toxicity test results and are used to demonstrate the health of the organisms used for toxicity testing.

9.8 Hardness, alkalinity, and ammonia in the overlying water typically should not vary by more than 50% during the sediment exposure, and dissolved oxygen should be maintained above 2.5 mg/L in the overlying water.

X. CALIBRATION

10.1 Instruments used for routine measurements of chemical characteristics such as pH, dissolved oxygen, temperature, and conductivity must be calibrated before use each day according to the instrument manufacturer's procedures and as indicated in GLEC's standard operating procedure for each instrument. See: LAB 1004, *The Calibration and Use of Liquid-in-Glass Bulb Thermometer and Digital Thermometers without Digital Correction Capabilities*; LAB 1036, *Use and Calibration of a Digi Sense (Barnant or Eutech)Thermocouple Thermometer;* LAB 1012, *Determination of pH in Water Samples Using the Orion 710A pH/ISE Meter and Thermo Scientific Orion pH Electrode*; LAB 1006, *Use of Dissolved Oxygen Meters*; and LAB 1010, *Use of Conductance of Aqueos Solutions Using YSI 33 or YSI 35 Conductivity Meters.*

10.2 Calibrate meters and probes in the morning (or before use) and again mid-day, or about every three hours when in use.

10.3 Calibrate the balance each day before use.

10.4 Calibration and maintenance data must be recorded in a permanent log specific to each meter.

XI. PROCEDURE

11.1 Analytical Procedure

11.1.1 Juvenile *Hyalella*, 7-8 days old are used to initiate the toxicity tests with laboratory reared or commercially purchased animals. (Test animals must be acclimated if reared in water different than the overlying water. The acclimation process is to first place animals in a 50/50 mixture of culture water and overlying water for 2 hours. They are then moved to a 25/75 mixture of culture water and overlying water for 2 hours followed by a transfer to 100% overlying water for 2 hours.)

11.1.2 *H. azteca* are fed 1 mL of YTC per 470 mL test chamber.

11.1.3 Test chambers are 470 mL glass (pint) jars, with screened drain holes at the 275 mL line, containing 100 mLs of sediment with 175 mLs of overlying laboratory water.

11.1.4 Juvenile *Hyalella* are placed in test chambers (470 mL beakers) using a large bore pipette, and exposed to test conditions for 28 or 42 days. Eight to sixteen replicates with ten organisms each are used for the control and test sediments depending on the study type/design. The overlying water is renewed manually a minimum of 2 times each day.

11.1.4.1 For the 28 day test endpoints, the sediment is sieved, the animals are removed, and survival is recorded. The surviving animals are then dried to a constant weight at 60° C then weighed on aluminum pans using an analytical balance. Dry weight is measured to five decimal places (0.00001g).

11.1.4.2 In 42 day test, the surviving organisms in the remaining replicates are transferred to water only beakers (each containing only overlying water and no sediment) on day 28. Survival and young production are monitored and recorded on day 35 and 42 of the test. On day 42, test termination, the surviving organisms are dried to a constant weight as described for the 28 day endpoint.

11.1.5 Prior to use, each sediment sample is homogenized to a uniform consistency and color. Sediment samples used for toxicity testing are stored in the dark at ≤ 6°C. The sediment is then added to each 470 ml glass test chamber by use of a stainless steel spatula or spoon. The overlying water is then added to each test chamber by pouring it into a water dispersing vessel held over the test chamber to avoid disturbing the sediment. The water and sediment are allowed to settle overnight before test animals are added.

11.1.6 Overlying water will be laboratory reconstituted water or dechlorinated water unless specified otherwise in a study plan.

11.1.7 The test concentrations will be undiluted whole sediment, control sediment and/or reference sediment.

11.1.8 Controls will be collected, stored, set up, and treated identically as the test treatments with regard to experimental conditions.

11.1.9 Ten *Hyalella* per test chamber are required for both the control and test concentrations.

11.1.10 If the dissolved oxygen concentration drops below 2.5 mg/L in any test chamber, the number of daily overlying water renewals will be increased for all replicates and treatments. Aeration could be used if frequent renewals (no more than four in a 24 hour period) do not raise the dissolved oxygen over 2.5 mg/L. The number of daily overlying water renewals will not exceed 4 times in a 24 hour period. Once the DO has increased above 3.0 mg/L; additional water renewals will be suspended until the DO falls again below 2.5 mg/L.

11.1.11 Lighting is provided by automatically controlled wide spectrum fluorescent lights (100-1000 lux) with a 16 hour light: 8 hour dark photoperiod.

11.1.12 Tests are conducted in a temperature controlled environmental chamber or water bath which maintains the water temperature in test chambers at 23 ± 1° C. A continuously operating recording thermometer provides a permanent record of the water temperature which is verified manually by daily manual temperature readings in alternating replicates throughout the test period.

11.1.13 Test Duration is 42 days or 28 days if reproduction endpoints are not necessary.

11.1.14 Endpoints are survival and growth for the 28 day exposure, while survival, growth and reproduction are the endpoints measured at the 42 day test termination. Dry weights are measured for *H. azteca* on day 28 and at test termination (day 42). Reproduction is monitored on days 35 and 42 of the test.

11.2 Water Quality Measurements

11.2.1 Hardness, alkalinity, and total ammonia are measured in the overlying water of each investigative sediment sample and control at the beginning, on day 28 and on day 41 for the 42 day test. Measure alkalinity, ammonia and hardness of each of the investigative and control samples by using a composite sample of overlying water taken from all replicates of each sample type (investigative or control). The composite sample will contain an equal amount of overlying water taken from each replicate to obtain approximately 200 mL. Temperature measurements are taken daily from two randomly selected replicate test chambers.

Conductivity is measured weekly. DO and pH are measured at least three times weekly from two randomly selected replicate test chambers.

11.2.1.1 Rinse water chemistry probes between replicates when measurements are directly from a test chamber to ensure that no organisms are attached to the probes.

11.2.1.2 Collect the overlying water for water quality measurements before water renewal, about 1 to 2 cm above the sediment surface. Make sure that no organisms are captured during collection in the pipet.

11.3 General Test Procedure

11.3.1 8 Days Before Test

11.3.2 Sieve culture to obtain 1-2 day old *H. azteca* and transfer them to a test organism holding chamber and feed (approximately 25 mLs of algae).

11.4 Day Before Test Initiation

11.4.1 Confirm that an adequate number of test organisms are available.

11.4.2 Thoroughly homogenize each investigative sediment samples and control sediments using a pre-cleaned stainless steel all purpose mixer and drill. The sediment is considered homogenized when the color and texture are uniform. Add 100 mL by volume of each sediment to each test chamber. Then add 175 mL of overlying water gently on top of the sediment in each test chamber. Allow the test chambers to settle for 24 hours. The daily overlying water renewal schedule begins at this time.

11.5 Start of Test (Day 0)

11.5.1 Measure hardness, alkalinity and ammonia using a composite sample of the overlying water from each replicate test chamber for each sediment type and each control. Collect equal amounts of water from each replicate to get approximately 200 mL for the composite sample. Measure temperature, pH, DO, and conductivity using two randomly selected replicates of each sediment type.

11.5.2 Create a random order of replicate test chambers and add 10 *Hyalella* to each replicate test chamber using a random numbers chart generated in an Excel spreadsheet. Archive at least 20-80 test organisms for initial dry weight determination. ***Note:*** *Replicate test chambers are selected at*

random by generating a random number for each replicate, then sorting the column of random numbers in ascending order, and finally adding the test organisms to each replicate in the order that they appear on the list.

11.5.3 Add food (1.0 mL YTC) to each replicate test chamber.

11.5.4 Observe organism behavior, brush drain hole screens, renew overlying water at specified times, and record observations on laboratory sheets.

11.5.5 Put test chambers in a temperature controlled environmental chamber or water bath set for 23 ± 1° C.

11.5.6 Document light intensity at a minimum of five locations over the area in which sediment toxicity testing is being completed (i.e. water bath) and record in the Light Metering Log Book. Record the ambient air temperature of the location (room) where the toxicity testing is being conducted.

11.6 Day 1-27

11.6.1 After each final daily renewal of overlying water, add 1.0 mL of YTC to each replicate test chamber. Measure temperature daily, conductivity weekly, and dissolved oxygen (DO) and pH three times/week, from 2 alternating replicates of each sediment type prior to water renewal. Observe behavior, mortality of test organisms and any other noteworthy characteristic (e.g., presence of other organisms, fungal or bacterial growths) of the sediment and/or overlying water and record on lab sheets daily.

11.7 Day 28

11.7.1 Measure all water quality parameters (temperature, pH, hardness, alkalinity, dissolved oxygen, conductivity, and total ammonia) prior to water renewal for each sediment type following the routine outlined in Section 11.2.1.

11.7.2 End the sediment-exposure portion of the test by collecting the test organisms with a #40 mesh sieve (425 µm mesh; U.S. standard size sieve). Randomly select four replicates for growth measurements (or as specified by the study plan) for the 42 day test: count survivors and preserve organisms in sugar formalin solution or oven dry immediately for growth measurements (see Section 11.12.2 and 11.12.4). ***Note:*** *The random selection of replicates is achieved by generating a random*

number for each of the 12 replicates, sorting the column of random numbers in ascending order, and finally selecting the first four replicates in the order that they appear on the list.

11.7.3 Use the remaining eight replicates for reproduction measurements: place surviving test organisms from each replicate into corresponding replicate water-only test chambers (300 mL high form lipless beaker with a screened drain hole at 175 mL). Each day, add 2 volume additions of overlying water and feed 1 mL YTC.

11.8 Reproduction Phase (Day 29-35)

11.8.1 Feed daily (1.0 mL YTC). Measure temperature daily, conductivity weekly, and DO and pH three times a week prior to water renewal from two randomly selected replicate test chambers. Measure hardness and alkalinity weekly from a composite sample of overlying water collected from each replicate. Observe behavior/mortality of test organisms.

11.9 Reproduction Phase (Day 35)

11.9.1 Record the number of surviving adults and remove, count and record the number of offspring. Return adults to their original individual beakers and add food.

11.10 Reproduction Phase (Day 36-41)

11.10.1 Feed daily (1.0 mL YTC). Measure temperature daily, conductivity weekly, and DO and pH three times a week prior to water renewal from two randomly selected replicates. Measure hardness and alkalinity weekly from a composite sample of all replicates. Observe and record behavior of test organisms.

11.11 Reproduction Phase (Day 41)

11.11.1 Measure all water quality parameters prior to overlying water renewal (pH, temperature, dissolved oxygen, hardness, alkalinity, conductivity, and ammonia) following the routine outlined previously.

11.12 Reproduction Phase (Day 42 Test Termination)

11.12.1 Check condition (live/dead) of test animals and record on data form.

11.12.2 On Day 42 sieve sediment from individual test chambers and collect surviving organisms. Determine the surviving number of adult female

Hyalella or preserve surviving adults in a sugar formalin solution to count the number of adults females at a later date. Once you have determined the number of adults females, take the weight of all of the surviving adults (see Section 11.12.4). The number of young, the total number of surviving adults, and the number of females in each replicate are counted and recorded on the data sheet.

11.12.3 The number of adult females is determined by simply counting the adult males. The number of adult males in each beaker is determined by sexing each of the surviving adults (from 11.12.2). This information is used to calculate the number of young produced per female per replicate from day 28 through day 42.

11.12.4 Place surviving test organisms in aluminum weighing pan (1 pan/replicate). Dry organisms to a constant weight at 60°C ± 4°C for 24 hours.

- Pan with dried test animals are brought to room temperature in a dessicator for a minimum of 30 minutes.
- Weigh pan with dried test animals to five decimal places (e.g., 0.00001 g) and record on data sheet.
- Remove dried test animals from pan, weigh pan only and record on data sheet.

11.12.5 The dried weight of *H. azteca* is determined by calculating the difference between the weight of the pan plus dried test animals and the weight of the pan minus dried test animals.

11.12.6 Dispose of sediment in prescribed manner, or according to the study plan.

11.12.7 Clean glassware and associated equipment and return to its proper location.

XII. DATA ANALYSIS AND CALCULATIONS

12.1 The endpoints measured in the 42 day tests include survival on days 28, 35, and 42 and growth (as length or dry weight) on days 28 and 42. Reproduction is the number of young/female produced from day 28 through 42. All data from the laboratory data forms are summarized in Excel spreadsheets. All endpoints will be analyzed using a statistical program (i.e., Toxcalc®, or Toxstat®) and following

the procedures described in Section 16 of the EPA/600/R-99/064, *Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates* (general information regarding statistical analysis of the data, including both point estimates (i.e., LC_{50}'s) and hypothesis testing (i.e., ANOVA).

12.2 Water quality data (i.e., pH, DO, conductivity, temperature, ammonia, alkalinity, hardness) are summarized by reporting the mean, range (min/max), and standard deviation for each treatment group (sediment type).

12.3 Biological endpoints (i.e., survival, growth, reproduction) are also summarized by reporting the mean, range, and standard deviation. A statistical analysis of the biological endpoints is also summarized by comparing treatment means and replicate variability to either laboratory or reference control means and replicate variability to determine statistical differences between them.

XIII. INSTRUMENT MAINTENANCE

13.1 pH probe internal filling and storage solution is changed weekly or sooner if the meter is not working properly.

13.2 Dissolved oxygen probe membrane and internal filling solution is changed monthly or sooner if the meter is not working properly.

13.3 Conductivity probe is cleaned at a minimum every month.

13.4 Temperature probes are checked against a NIST thermometer every quarter or as requested by the study plan.

13.5 Other instruments used will be maintained following the manufacturer's recommendations and the appropriate standard operating procedure.

XIV. QUALITY ASSURANCE

14.1 Records will be maintained by the laboratory coordinator. Hard copies of all data generated or acquired will be kept in secure files at GLEC. All electronic data or other information will be filed and stored by the project name on a shared computer server which is back-upped daily. Original file copies of data are always kept in-house at GLEC. Duplicate copies of information will be produced if it is necessary for the data to leave the GLEC offices.

14.2 All raw data is reviewed using a three-tiered review process. After the raw data has been summarized in an excel format, it is reviewed by a peer for completeness and accuracy and the final review is a ten percent review of all the data. If any

errors or omissions are observed during the ten percent review, the entire data set is reviewed again.

14.3 The toxicology laboratory technical supervisor generates the report. After the report is originated, it is reviewed by a second toxicology staff member. This review evaluates the computations performed, and the accuracy and traceability of the data. It is the responsibility of the person who generated the draft report to satisfactorily address any of the reviewer's comments and concerns and to generate the final report.

14.4 The final review is conducted by the GLEC Manager of Operations or a qualified GLEC upper level staff member before the report is submitted to the client.

XV. WASTE MANAGEMENT

When testing is completed the sediment will be collected and disposed of by a certified chemical waste company or returned to the client as per the study plan.

XVI. DEVIATIONS

16.1 Test duration may be shortened to evaluate survival and growth endpoints at 28-days.

16.2 Any deviation to this SOP must be accurately documented in the laboratory log books and on the toxicity test data forms.

XVII. REFERENCES

17.1 ASTM 1706-95b, *Standard Test Methods for Measuring the Toxicity of Sediment associated Contaminants with Fresh Water Invertebrates;* and ASTM 1391-94, *Standard guide for Collection, Storage, Characterization, and Manipulation of Sediments for Toxicological Testing.*

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

17.3 GLEC SOP LAB 1004, The Calibration and Use of Liquid-in-Glass Bulb Thermometer and Digital Thermometers without Digital Correction Capabilities.

17.4 GLEC SOP LAB 1006, Use of Dissolved Oxygen Meters.

17.5 GLEC SOP LAB 1010, Use of Conductance of Aqueos Solutions Using YSI 33 or YSI 35 Conductivity Meters.

17.6 GLEC SOP LAB 1012, Determination of pH in Water Samples Using the Orion 710A pH/ISE Meter and Thermo Scientific Orion pH Electrode.

17.7 GLEC SOP LAB 1036, Use and Calibration of a Digi Sense (Barnant or Eutech) Thermocouple Thermometer.

17.8 GLEC SOP TOX 1020, Manual Cleaning of Glassware and Equipment Used in Toxicity Tests.

17.9 TOXSTAT program version 3.5, 1996.

STANDARD OPERATING PROCEDURE FOR LONG TERM WHOLE SEDIMENT TOXICITY TESTS WITH *Chironomus dilutus*

SED 7004

Method Reference: ASTM 1706-95B AND 1391-94 AND EPA/600/R-99/064

January 2012

Great Lakes Environmental Center, Inc.
(GLEC)

Mailee W. Garton — 1/9/2012
Mailee Garton — Date
Technical Author

Michelle VanDenBrand — 1/9/12
Michelle VanDenBrand — Date
User Reviewer

Dennis J McCauley — 1/9/12
Dennis McCauley — Date
President/Principal Research Scientist

Jennifer Hansen — 1/9/12
Jennifer Hansen — Date
GLEC Quality Assurance Officer

TABLE OF CONTENTS

I. SCOPE AND APPLICATION

1.1 This SOP describes the methodology for conducting long-term or life cycle (50-65 days), whole sediment toxicity tests with *Chironomus dilutus*. Test duration may be shortened to 20 days to evaluate only long-term survival and growth endpoints. After 20 days, the test organisms are expected to initiate transformation to the pupae stage. Consequently, a 20 day exposure only evaluates contaminated sediment effects on larvae survival and growth, whereas, the longer term tests (50-65 days) evaluate contaminated sediment effects on emergence, long-term survival, and reproduction.

1.2 *C. dilutus* is a commonly reared insect in a toxicology laboratory setting and is one of two principal test organisms used to measure the toxicity of freshwater sediment.

II. SUMMARY OF METHOD

2.1 During this test, test organisms are continuously exposed for either 20 days or up to 50-65 days to sediment with intermittent renewal of overlying water. *Chironomus* survival, growth, emergence, and reproduction endpoints are recorded at the end of a 50-65 day testing period. Only larvae survival and growth can be measured after a 20 day exposure period. The survival, growth, emergence, and reproduction data are used to determine if the whole sediment is toxic (measured by reduced survival, growth, emergence, or reproduction) relative to a suitable reference control or laboratory control sediment.

2.2 This SOP is based on *Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates*, EPA/600/R-99/064, ASTM 1706-95b, *Standard Test Methods for Measuring the Toxicity of Sediment associated Contaminants with Fresh Water Invertebrates;* and ASTM 1391-94, *Standard guide for Collection, Storage, Characterization, and Manipulation of Sediments for Toxicological Testing.*

III. DEFINITIONS

3.1 Ash Free Dry Weight (AFDW) - The AFDW of the test animals is determined by calculating the difference between the weight of the pan with dried test animals and the weight of the pan plus the ashed test animals.

3.2 Contaminated sediment – Sediment containing chemical substances at concentrations that may pose a known or suspected threat to aquatic life, or human health (the test materials/samples for this method).

3.3 Control sediment – An uncontaminated sediment (e.g., "clean" sediment collected in the field from a known contaminant free water body or formulated sediment that is prepared in the laboratory) that is essentially free of contaminants and is used routinely to assess the acceptability of a test and the health of the test organism.

3.4 Dechlorinated tap water - Municipal tap water is dechlorinated at GLEC using carbon-bed tanks. The dechlorinated water is checked weekly in the laboratory, and must contain less than or equal to .02 ppm of chlorine.

3.5 Deionized (DI) water – Water that has had its mineral ions removed. It is produced by purifying tap water by reverse osmosis followed by passing it through carbon and de-ionization cartridges. It is a physical process using ion exchange resins which bind to and filter out the mineral salts from water. (GLEC's DI Water meets Type II Reagent Water specifications).

3.6 Formulated sediment – Mixtures of clay, sand and organic materials used to mimic the physical components of natural sediment.

3.7 Sediment – Particulate soil material that usually lies below water and formulated soils that are intended to lie below water in a whole sediment toxicity test.

3.8 Whole sediment – Field collected sediment and associated pore (interstitial water) water that have had minimal manipulation or disturbance.

3.9 Overlying water – water that is placed over sediment in a test chamber during a test. Usually dechlorinated water, but depending on the study plan may be surface water, or water provided by the client.

3.10 Reconstituted water - Synthetic dilution water, prepared with deionized water and reagent grade chemicals or mineral water, to achieve measurable water quality values of pH, hardness, and alkalinity.

3.11 Reference sediment – A sample of uncontaminated whole sediment collected from an area near the area of concern used to assess sediment conditions exclusive of material(s) of interest.

IV. INTERFERENCES AND CAUTIONS

4.1 Interferences are characteristics of a sediment or sediment test system, aside from those related to sediment-associated chemicals of concern that can potentially affect test organism survival, growth, or reproduction.

4.2 Interferences can potentially confound interpretation of test results in two ways: (1) false-positive response, i.e., toxicity is observed in the test when

contamination is not present at concentrations known or suspected to elicit a response, or there is more toxicity than expected; and (2) false-negative response, i.e., no toxicity or bioaccumulation is observed when contaminants are present at concentrations that are known or suspected to elicit a response, or there is less toxicity or bioaccumulation than expected.

4.3 Interfering factors can cause false-negative or false-positive responses:

4.3.1 Physical and chemical characteristics of sediment affecting toxicity independent of chemical concentration (i.e., non-contaminant factors), such as grain size, acid volatile sulfides, total organic carbon, and redox potential.

4.3.2 Changes in chemical bioavailability as a function of sediment manipulation or storage, such as aeration, volatilization, reduction, and oxidation.

4.3.3 The presence of predaceous indigenous organisms.

4.4 Equipment and supplies that contact sediment and overlying water should be chosen to minimize sorption of test materials from the sediment and must not add toxicity. Glass, type 316 stainless steel, nylon, high-density polyethylene, polypropylene, polycarbonate, and fluorocarbon plastics should be used whenever possible to minimize leaching, dissolution, and sorption.

V. HEALTH AND SAFETY

5.1 Each whole sediment sample should be regarded as a potential health hazard and exposure should be as low as reasonably achievable.

5.2 Technicians must wear appropriate protective clothing, at a minimum a lab coat, safety glasses and disposable (latex or nitrile) gloves, while working near the samples during the testing period, while disposing of test materials and cleaning glassware or equipment.

VI. EQUIPMENT AND SUPPLIES

6.1 Analytical balance capable of accurately weighing to 0.01 mg.

6.2 Aluminum weigh boats.

6.3 Carbouy.

6.4 Client Sediment Log Book.

6.5 Temperature controlled refrigerator units.

6.6 Desiccator.

6.7 Dissecting microscope.

6.8 Droppers, and glass tubing with fire polished edges, 4- to 6-mm ID – for transferring test organisms.

6.9 Dissolved oxygen (DO), pH/selective ion, ammonia, and specific conductivity meters and probes for routine chemical measurements are needed.

6.10 Drying oven.

6.11 *Chironomus* egg cases or known age larvae.

6.12 Emergence traps.

6.13 Temperature controlled environmental chamber or water bath.

6.14 Forceps.

6.15 Juvenile test organism holding beakers.

6.16 Lab hood or sample handling ventilation system.

6.17 Larval test organism rearing chambers.

6.18 Light box.

6.19 Light meter.

6.20 Thermometer, calibrated.

6.21 Nitex screen 60 micron.

6.22 Pipets and bulbs.

6.23 Shallow glass or plastic pans.

6.24 Sample Check-In Log Book (#24).

6.25 Test chambers – 470 mL (pint) glass jars with nylon screened drain hole measured at 275 mL.

6.26 Thermometer – handheld and temperature data logger.

6.27 Reference weights, Class 4 for documenting the performance of the analytical balance(s). The balance(s) should be checked with reference weights that are at the upper and lower ends of the range of the weightings made when the balance is used.

6.28 Serological pipets – 1 to 10 mL, graduated.

6.29 Stainless steel bowls and spoons.

6.30 Tetrafin Slurry 4mg/L – TFS Log Book (Log #9).

6.31 Volumetric flasks and graduated cylinders – Class A, borosilicate glass or nontoxic plastic labware10 to 1000 mL for making test solutions.

6.32 Wash bottles – filled with DI water for rinsing small glassware, instrument electrodes, and probes.

VII. REAGENTS AND STANDARDS

7.1 Gases – Not Applicable. No gases are used in this procedure.

7.2 Water –

7.2.1 DI Water

7.2.2 Dechlorinated water

7.2.3 Reconstituted water

7.2.4 Other – as needed as per study plan for overlying water that is other than dechlorinated water.

7.3 Reagents – Not Applicable. No reagents are used in this procedure.

7.4 Standard Solutions – Not Applicable. No standard solutions are used in this procedure.

7.5 Biological Specimens – *Chironomus dilutus.*

VIII. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 Sediment samples to be tested are stored in the dark at $\leq 6^{\circ}C$ until used. Besides keeping samples refrigerated and dark, no other preservation is required.

8.2 GLEC SOP's for further information: TOX 0001, *Standard Operating Procedure for Storage and Handling of Test Materials;* SED 7002, *Standard Operating Procedure for Whole Sediment Sample Collection and Shipment for Toxicological Tests.*

IX. QUALITY CONTROL

9.1 Records will be maintained as indicated in this SOP. These are reviewed by the study director and/or supervisory personnel.

9.2 Glassware and associated equipment will be cleaned in the prescribed manner (GLEC SOP #TOX 1020 *Manual Cleaning of Glassware and Equipment Used in Toxicity Tests*) and returned to the appropriate location.

9.3 *Chironomus dilutus* – Minimum mean control survival must be 70% or greater on day 20.

9.4 *Chironomus dilutus* – Minimum growth of at least 0.6 mg/ surviving organism as dry weight or 0.48 mg/surviving organism as AFDW (measured as dry weight) at day 20.

9.5 *Chironomus dilutus* – Emergence should be greater than or equal to 50% in the laboratory control sediment test organisms.

9.6 *Chironomus dilutus* – Time to death after emergence should be <6.5 days for males and <5.1 days for females in the laboratory control sediment test organisms.

9.7 *Chironomus dilutus* – The mean number of eggs/egg cases should be greater than or equal to 800 and the percent hatch should be greater than or equal to 80% in the laboratory control sediment test organisms.

9.8 If any of the above criteria are not met, the laboratory supervisor will contact the client. Depending on the client instruction, the test may have to be re-initiated.

9.9 Archive 20-80 test organisms on day 10 for initial weight or length determination.

9.10 Reference toxicant testing is conducted using sodium chloride as the toxicant with each batch of organisms purchased from an outside source, used to initiate a test. Otherwise, test organisms cultured in house will be tested at test initiation or during the month the sediment test is conducted. Control charts generated from the reference toxicity testing results are provided with toxicity tests and are used to demonstrate the health of the organisms used for toxicity testing.

9.11 Hardness, alkalinity, and ammonia in the overlying water typically should not vary by more than 50% during the sediment exposure, and dissolved oxygen should be maintained above 2.5 mg/L in the overlying water.

X. CALIBRATION

10.1 Instruments used for routine measurements of chemical characteristics such as pH, dissolved oxygen, temperature, and conductivity must be calibrated before use each day according to the instrument manufacturer's procedures and as indicated in GLEC's standard operating procedure for each instrument. See: LAB 1004, *The Calibration and Use of Liquid-in-Glass Bulb Thermometer and Digital Thermometers without Digital Correction Capabilities*; LAB 1036, *Use and Calibration of a Digi Sense (Barnant or Eutech)Thermocouple Thermometer;* LAB 1012, *Determination of pH in Water Samples Using the Orion 710A pH/ISE*

Meter and Thermo Scientific Orion pH Electrode; LAB 1006, *Use of Dissolved Oxygen Meters*; and LAB 1010, *Use of Conductance of Aqueos Solutions Using YSI 33 or YSI 35 Conductivity Meters.*

10.2 Calibrate meters and probes in the morning (or before use) and again mid-day, or about every three hours when in use.

10.3 Calibrate the balance each day before use.

10.4 Calibration and maintenance data must be recorded in a permanent log specific to each meter.

XI. PROCEDURE

11.1 Test System

11.1.1 Laboratory reared *C. dilutus*, < 24 hours old are used to initiate the toxicity tests.

11.1.2 *C. dilutus* are fed 1.5 mL of a 4 g/L suspension of Tetrafin® goldfish food in deionized water, per 470 ml jar.

11.1.3 Test chambers are 470 ml (pint) glass jars containing 100 ml of sediment with approximately 175 ml of overlying laboratory water.

11.1.4 Newly hatched (<24 hr.) *C. dilutus* are randomly placed in test chambers (470 ml jars) by locating the hatching young with a dissecting microscope and transferred to the test chambers using a glass Pasteur pipette. **Note:** Randomization is achieved by generating a random number for each replicate number, and sorting the list of random numbers using an Excel spreadsheet. This results in a randomized list of replicate numbers. Test organisms are added to the replicate listed at the top of the list first, then sequentially after that. The organisms are exposed to test conditions until there is no recorded emergence in a given treatment for seven consecutive days (approximately 50-65 days) unless otherwise specified in a study plan. Sixteen replicates (12 are initiated on day 0 and 4 replicates for auxiliary males are typically initiated on day 10) with twelve organisms each, are initiated for the control and test sediment, or as designated by a specific study design.

11.1.5 The test may be terminated at 20 days if larvae survival and growth are only of interest. There are typically a minimum of eight replicates in a 20 day test design.

11.1.6 The overlying water is renewed manually a minimum of 2 times each day or at least once every 12 hours in each replicate test chamber.

11.1.7 The test is ended separately for each sediment type between days 50-65, when no additional emergence has been recorded for seven consecutive days. If no emergence is recorded from a treatment for a period of seven consecutive days, the treatment may be terminated when the control sediment has had no emergence for seven consecutive days.

11.1.8 The endpoints include 20 day survival and growth, weight gain; long term survival, female and male emergence, adult mortality, the number of egg cases oviposited, the number of eggs produced and the number of hatched eggs. In 20 day tests, only larvae survival and growth are measured.

11.1.9 Sediment samples to be tested are stored in the dark at 4° C (0-6 ˚C) until used. The sediment is then added (100 mL/beaker) to 470 mL jars by use of a stainless steel spatula or spoon. The overlying water is then added to the beaker by pouring it into a water dispersing vessel held over the test chamber to avoid disturbing the sediment. The water and sediment are allowed to settle overnight before test animals are added.

11.1.10 Overlying water may be dechlorinated laboratory water unless specified otherwise in a study plan.

11.1.11 The test concentrations will be undiluted whole sediment, control sediment and/or reference sediment.

11.1.12 Controls will be set up and treated identically as the treatment chambers with regard to experimental conditions.

11.1.13 Twelve *C. dilutus* per chamber are required for both the control and test concentrations.

11.1.14 If dissolved oxygen (DO) drops below 2.5 mg/L in one treatment and fungal/bacterial growths are noted on the sediment surface, feeding should be suspended in all treatments until the DO rises above 2.5 mg/L. If no surface growths are observed, the number of daily overlying water renewals will be increased (up to 4 times per day) for all treatments until the DO recovers to greater than 2.5 mg/L. Aeration may be used if frequent renewals and suspending food additions do not remedy the low dissolved oxygen.

11.1.14.1 Once DO has increased above 3.0 mg/L; additional water renewals will be suspended, until DOs fall below 2.5 mg/L.

11.1.15 Lighting is provided by wide spectrum fluorescent lights (100-1000 lux) with an automatically controlled 16 hour light: 8 hour dark photoperiod.

11.1.16 Tests are conducted in a temperature controlled environmental chamber or water bath which maintains the water temperature in test chambers at 23±1° C. A continuously operating recording thermometer provides a permanent record of the water temperature and the water temperature is also manually checked daily during the test period.

11.1.17 Test duration is approximately 50-65 days unless otherwise specified.

11.1.18 Endpoints are 20 day survival and growth, or survival, growth, emergence and reproduction for the long-term test. Ash free dry weights (AFDW) are measured for *C. dilutus* at test termination. Reproduction or other sublethal endpoints are monitored on days 40 through test termination.

11.2 Water Quality Measurements

11.2.1 Hardness, alkalinity, and total ammonia are measured in the overlying water on Day 0, at day 20 and at the end of the test from a composite sample of each investigative sediment and each control. The composite sample will contain an equal amount of overlying water from each replicate to obtain approximately 200 mLs. Temperature measurements are taken daily from two randomly selected replicate test chambers of each investigative sediment and each control. Conductivity is measured weekly and DO and pH are measured at least three times per week from two randomly selected replicate test chambers (of each investigative sediment and each control). The number of DO measurements will be increased if the DO drops more than 1 mg/L in one day, or the DO falls below 2.5 mg/L.

11.3 General Test Procedure

11.3.1 Four Days Before Test

11.3.1.1 Initiate test organism reproduction flask with cultured adults with a 1:3 male: female ratio.

11.3.2 Three Days Before Test

11.3.2.1 Collect egg cases (6-8 egg cases) and incubate at 23°C in a shallow pan, filled with laboratory water.

11.3.3 Two Days Before Test

11.3.3.1 Check egg cases for viability and development.

11.3.4 Day Before Test

11.3.4.1 Check egg cases for hatch and development.

11.3.4.2 The investigative sediment samples and control sediment are thoroughly homogenized, using a clean stainless steel all-purpose mixer and drill. The sediment is considered homogenized when the color and texture of the sample are uniform. Add 100 mL by volume of sediment is added to each test chamber. Overlying water is also added to each test chamber. The test chambers are then allowed to settle for 1 hour; after 1 hour has passed, 1.5 mL of Tetrafin slurry is added to each replicate chamber. The all purpose mixer is washed and dried between each type of sediment being homogenized. Record observations in the Client Sediment Log Book.

11.3.5 The daily renewal schedule of overlying water begins at this time.

11.3.6 Start of Test (Day 0)

11.3.6.1 Transfer *C. dilutus* egg cases to a crystallizing dish containing control water. (Discard any larvae that have already left the egg cases in the incubation dishes).

11.3.6.1.1 Larvae hatched after egg case transfer are <24 hours and will be used to initiate the tests.

11.3.6.2 Hardness, alkalinity, conductivity, pH, dissolved oxygen, temperature, and ammonia are measured for each sediment type. Temperature, pH, DO, and conductivity are measured from two randomly selected replicates (of each investigative sediment and each control). Alkalinity, hardness, ammonia and are measured of each investigative sediment and each control from a composite sample of all test chamber replicates.

11.3.6.3 Pour off half of the overlying water from each test chamber and add 1.5 ml of Tetrafin slurry to each test chamber.

11.3.6.4 Twelve *C. dilutus* larvae (< 24 hours old) are added to each beaker; test chambers should not set outside the temperature controlled test system for no more than an hour.

11.3.6.4.1 Larvae are added randomly using a randomized number chart generated in Microsoft Excel.

11.3.6.5 Observe organism behavior and record on daily laboratory sheets.

11.3.6.6 Test chambers are put in a temperature controlled environmental chamber or water bath at 23 ± 1° C.

11.3.6.7 Light intensity is documented at a minimum of five locations over the area in which sediment toxicity testing is being completed (i.e. water bath).

11.3.7 Day 1 through test termination

11.3.7.1 On a daily basis, add 1.5 mL of food to each test replicate chamber. Measure temperature daily, conductivity weekly, and dissolved oxygen (DO) and pH three times weekly, from 2 alternating replicates of each sediment type prior to water renewals. Observe behavior of test organisms and record on lab sheets daily (Attachment 1).

11.3.8 Day 6

11.3.8.1 Follow set-up schedule for auxiliary male beakers (4 replicates per treatment) described above for day -3 to day 0 if the test includes long-term emergence and reproduction endpoints.

11.3.9 Day 19

11.3.9.1 Ash weigh pans at 550°C for 2 hours for Day 20 weight determinations.

11.3.10 Day 20 - Survival and Growth Endpoint

11.3.10.1 Hardness, alkalinity, conductivity, pH, dissolved oxygen, temperature, and ammonia are measured for each sediment type. Temperature, pH, DO, and conductivity are measured from two randomly selected replicates (of each investigative sediment and each control). Alkalinity, hardness, ammonia

and are measured of each investigative sediment and each control from a composite sample of all test chamber replicates.

11.3.10.2 Randomly select four of the twelve replicates from each treatment to recover larvae for growth and survival determination. Pool all living larvae per replicate and dry the sample to a constant weight at 60°C for 24 hours.

11.3.10.3 The four randomly selected replicates are determined by a randomization chart generated in Microsoft Excel. **Note:** the random selection of replicates is achieved by generating a random number for each replicate (1-12), sorting the column of random numbers in ascending order, and finally selecting the first four replicates from the list.

11.3.10.4 If the test is designed as a 20 day survival and growth only test, recover larvae from all replicates for growth and survival determination.

11.3.10.5 Install emergence traps on each of the remaining replicate test chambers for the long-term toxicity test.

11.3.11 Day 21

11.3.11.1 The dried larvae samples are brought to room temperature in a desiccator (minimum of 30 minutes) and weighed to the nearest 0.01 mg (five decimal places, e.g., 0.00001g), and recorded on a data form. The dried larvae in the pan are then ashed at 550°C for 2 hours then placed in desiccator for a minimum 30 minutes. The pan with the ashed larvae is then weighed again and recorded on a data form. The tissue mass of the larvae is determined by calculating the difference between the weight of the dried larvae plus pan and the weight of the ashed larvae plus pan.

11.3.12 Day 23 – Emergence and Reproduction Monitoring

11.3.12.1 On a daily basis, count and record the emergence of males and females, pupal, and adult mortality, and time to death for previously collected adults. Each day, transfer the newly emerged adults from each replicate to a corresponding reproduction/oviposition (R/O) chamber. As egg masses are produced in each R/O chamber, transfer each primary egg case from the R/O chamber to a corresponding petri dish. At this

time, depending on the study plan, observations on incubation and hatchability, and/or estimates on the number of eggs in the primary egg cases can be completed.

11.3.13 Day 28

11.3.13.1 Place emergence traps on auxiliary male replicate test chambers.

11.3.14 Day 33

11.3.14.1 Transfer males emerging from the auxiliary male test chambers to individual inverted petri dishes. The auxiliary males are used for mating with females from corresponding treatments from which most of the males had already emerged or in which no males emerged.

11.3.15 Day 40 – Test Termination

11.3.15.1 Measure temperature daily, conductivity weekly, and dissolved oxygen (DO) and pH three times weekly, from 2 alternating replicates of each sediment type prior to water renewals.

11.3.15.2 After 7 days (or according to a study plan) of no recorded emergence in a given treatment, end the treatment by sieving the sediment to recover remaining larvae, pupae or pupal exuviae. When no emergence occurs in a test treatment, that treatment can be ended once emergence in the control sediment has ended using the 7 day or alternate criterion.

11.3.15.3 Measure hardness, alkalinity, conductivity, pH, dissolved oxygen, temperature, and ammonia are for each sediment type at test termination. Measure temperature, pH, DO, and conductivity from two randomly selected replicates. Alkalinity, hardness, ammonia and are measured from a composite sample of all test chamber replicates.

11.3.15.4 Dispose of sediment as specified by Project Manager (i.e., collect and return to client or collect and dispose of using a certified chemical waste company).

11.3.15.5 Collect dirty glassware and associated equipment in the gray toxicology bins and deliver to the dish room.

XII. DATA ANALYSIS AND CALCULATIONS

12.1 The endpoints measured in the life cycle test include survival at Day 20 and at about 50-65 days (end of the test), growth (as Ash-Free-Dry-Weight – (AFDW)) on day 20, and emergence and reproduction are monitored after Day 23 through the end of the test. Reproduction is the number of eggs/egg cases, which are incubated for 6 days to determine hatching success. Each investigative sediment is ended separately when no additional emergence has been recorded for seven consecutive days (unless otherwise stated in a project study plan). All data from the laboratory data forms are summarized in Excel spreadsheets. All endpoints are analyzed using a statistical program (i.e., Toxcalc or Toxstat®) and following the procedures described in Section 15 and 16 of the EPA/600/R-99/064, Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates (general information regarding statistical analysis of the data, including both point estimates (i.e., LC50s) and hypothesis testing (i.e., ANOVA)).

12.2 Water quality data (i.e., pH, DO, conductivity, temperature, ammonia, alkalinity, hardness) are summarized by reporting the mean, range (min/max), and standard deviation for each treatment group (sediment type).

12.3 Biological endpoints (i.e., survival, growth, reproduction, and emergence) are also summarized by reporting the mean, range, and standard deviation. A statistical analysis of the biological endpoints is also summarized by comparing treatment means and replicate variability to either laboratory or reference control means and replicate variability to determine statistical differences between them.

XIII. INSTRUMENT MAINTENANCE

13.1 pH probe internal filling and storage solution is changed weekly or sooner if the meter is not working properly.

13.2 Dissolved oxygen probe membrane and internal filling solution is changed monthly or sooner if the meter is not working properly.

13.3 Conductivity probe is cleaned at a minimum every three months.

13.4 Temperature probes are checked against a NIST thermometer every quarter, as outlined in their SOP, or as requested by a specific study plan.

13.5 Other instruments used will be maintained following the appropriate standard operating procedure.

XIV. QUALITY ASSURANCE

14.1 Records will be maintained by the laboratory coordinator. Hard copies of all data generated or acquired will be kept in secure files at GLEC. Any electronic data or other information will be filed and stored by the project name on a shared computer server which is back up daily. Original file copies of data are always kept in-house at GLEC. Duplicate copies of information will be produced if it is necessary for the data to leave the GLEC offices.

14.2 All raw data, is reviewed using a three-tiered review. After the raw data has been summarized in an excel format, it is reviewed by a peer for completeness and accuracy and the final review is a ten percent review of all the data. If any errors or omissions are observed during the ten percent review, the entire data set is reviewed again.

14.3 The toxicology laboratory technical supervisor generates the report. After the report is originated, it is reviewed by a second toxicology staff member. This review evaluates the computations performed, and the accuracy and traceability of the data. It is the responsibility of the person who generated the report to ssatisfactorily address any of the QA reviewer's comments and concerns and to generate the final report.

14.4 The final review is conducted by the GLEC Manager of Operations or a qualified GLEC upper level staff member before the report is submitted to the client.

XV. WASTE MANAGEMENT

When testing is completed the sediment will be collected and disposed of by a certified chemical waste company, or returned to the client as per the study plan.

XVI. DEVIATIONS

16.1 Test duration may be shortened to evaluate survival and growth endpoints at 20 days. Procedure is the same with the exception of using 8 to 12 replicates per investigative sediment dependent of study plan.

16.2 Any deviation to this SOP must be accurately documented in the laboratory log books, on the toxicity test data forms, and in reports to clients.

XVII. REFERENCES

17.1 ASTM 1391-94, *Standard Guide for Collection, Storage, Characterization, and Manipulation of Sediments for Toxicological Testing.*

17.2 ASTM 1706-95b, *Standard Test Methods for Measuring the Toxicity of Sediment-Associated Contaminants with Fresh Water Invertebrates.*

17.3 EPA/600/R-99/064. Methods for Measuring the Toxicity and Bioaccumulation of Sediment-Associated Contaminants with Freshwater Invertebrates.

17.4 GLEC SOP LAB 1004, The Calibration and Use of Liquid-in-Glass Bulb Thermometer and Digital Thermometers without Digital Correction Capabilities.

17.5 GLEC SOP LAB 1006, Use of Dissolved Oxygen Meters.

17.6 GLEC SOP LAB 1010, Use of Conductance of Aqueos Solutions Using YSI 33 or YSI 35 Conductivity Meters.

17.7 GLEC SOP LAB 1012, Determination of pH in Water Samples Using the Orion 710A pH/ISE Meter and Thermo Scientific Orion pH Electrode.

17.8 GLEC SOP LAB 1036, Use and Calibration of a Digi Sense (Barnant or Eutech) Thermocouple Thermometer.

17.9 GLEC SOP SED 7002, Standard Operating Procedure for Whole Sediment Sample Collection and Shipment for Toxicological Tests.

17.10 GLEC SOP TOX 0001, Standard Operating Procedure for Storage and Handling of Test Materials.

17.11 GLEC SOP TOX 1020, Manual Cleaning of Glassware and Equipment Used in Toxicity Tests.

17.12 TOXCALC program version 5.0.32. Copyright 1994-2009.

17.13 TOXSTAT program version 3.5, 1996.

Attachment

Laboratory Data Forms

Chironomus dilutus
Long-Term Whole Sediment Toxicity Tests

Sample ID:

Chironomus dilutus Long Term Survival, Growth, and Hatch Whole Sediment Toxicity Test

GLC#:
Sample ID:
Test Species: *Chironomus dilutus*
Date Addition of Sediment:
Test Initiation Date:

Test Day: Day 0
Date:
Overlying Water:
Overlying Water Batch ID (GLC Number):
□ chemistries time/Initial

Test Photoperiod: 16:8
Test System: 175mL Manual Delivery
Test Temperature: 23± 1°C
Test Organism Source/Age:
Test Termination Date:

Number Daily Renewals: 2
□ renewal time/Initials □ renewal time/Initials
□ renewal time/Initials □ renewal time/Initials
Food: TFS (Tetrafin 4g/L)# □ Feed 1.5 ml/replicate
Screens Cleaned: □ yes □ no □ n/a

Replicate	Temperature (23± 1°C)*	pH	Dissolved Oxygen (mg/L)*	Specific (µmhos/cm)	Hardness mg/L $CaCO_3$	Alkalinity mg/L $CaCO_3$	Ammonia (as N)	Observations/ 10 organisms per replicate
1								
2								
3					end:	end:		
4					start:	start:		
5					Titrant	Titrant		
6					used (mL):	used (mL):		
7					Sample	Sample		
8					volume (mL):	volume (mL):		
9								
10								
11								
12								

* Contact Laboratory Coordinator if Dissoved Oxygen level is < 2.5 mg/L or Temperature is out of range.
*Alkalinity, hardness and ammonia analyzed from a composite sample of all 8 replicates.
*If DO declines by more thatn 1.0 mg/L since previous measurement, then measure daily.

KEY: AV: Animals Visible
NAV: No Animals Visible
FOV: Foreign Organism Visible
BHV: Burrow Holes Visible

Relative % Difference: RPD ≤15%

$$RPD = \left| \frac{(s_1 - s_2)}{(s_1 + s_2)/2} \right| \times 100 =$$

Ammonia Reporting Limits:
RL = Reporting Limit (0.10 mg/L).
MDL = Minimum Detection Limit (0.04 mg/L) - last updated 11/15/10.
J = ≥ MDL and < RL.
U = Below MDL.

Sample ID:

QC'd by:_____

Chironomus dilutus Long Term Survival, Growth, and Emergence Whole Sediment Toxicity Test

GLC#:	Test Photoperiod: 16:8
Sample ID:	Test System: 175mL Manual Delivery
Test Species: *Chironomus dilutus*	Test Temperature: 23± 1°C
Date Addition of Sediment:	Test Organism Source/Age:
Test Initiation Date:	Test Termination Date:

Test Day:	Test Day:
Date:	Date:
Overlying Water:	Overlying Water:
Overlying Water Batch ID (GLC Number):	Overlying Water Batch ID (GLC Number):
Number Daily Renewals:	Number Daily Renewals:
□ renewal time/Initials □ renewal time/Initials	□ renewal time/Initials □ renewal time/Initials
□ renewal time/Initials □ renewal time/Initials	□ renewal time/Initials □ renewal time/Initials
Food: TFS (Tetrafin 4g/L)# □ Feed 1.5 ml/replicate	Food: TFS (Tetrafin 4g/L)# □ Feed 1.5 ml/replicate
Screens Cleaned: □ yes □ no □ n/a	Screens Cleaned: □ yes □ no □ n/a

□ chemistries time/Initial

Replicate	Temperature (23± 1°C)*	Dissolved Oxygen (mg/L)*	Observations
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			

□ chemistries time/Initial

Replicate	Temperature (23± 1°C)*	Dissolved Oxygen (mg/L)*	pH	Observations
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				

Key: AV: Animals Visible NAV: No Animals Visible FOV: Foreign Organism Visible BHV:Burrow Holes Visible

* Contact Laboratory Coordinator if Dissoved Oxygen level is < 2.5 mg/L or Temperature is out of range.

*If DO declines by more than 1.0 mg/L since previous measurement, then measure daily.

QC'd by:_____

Chironomus dilutus Long Term Survival, Growth, and Emergence Whole Sediment Toxicity Test

GLC#:	Test Photoperiod: 16:8
Sample ID:	Test System: 175mL Manual Delivery
Test Species: *Chironomus dilutus*	Test Temperature: 23± 1°C
Date Addition of Sediment:	Test Organism Source/Age:
Test Initiation Date:	Test Termination Date:

Test Day: Day

Date:

Overlying Water:

Overlying Water Batch ID (GLC Number):

Number Daily Renewals:

□ renewal time/Initials □ renewal time/Initials

□ renewal time/Initials □ renewal time/Initials

Food: TFS (Tetrafin 4g/L)# □ Feed 1.5 ml/replicate

Screens Cleaned: □ yes □ no □ n/a

□ chemistries time/Initial

Replicate	Temperature (23± 1°C)*	Dissolved Oxygen (mg/L)*	Observations
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			

Test Day: Day

Date:

Overlying Water:

Overlying Water Batch ID (GLC Number):

Number Daily Renewals:

□ renewal time/Initials □ renewal time/Initials

□ renewal time/Initials □ renewal time/Initials

Food: TFS (Tetrafin 4g/L)# □ Feed 1.5 ml/replicate

Screens Cleaned: □ yes □ no □ n/a

□ chemistries time/Initial

Replicate	Temperature (23± 1°C)*	Dissolved Oxygen (mg/L)*	Observations
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			

Key: AV: Animals Visible NAV: No Animals Visible FOV: Foreign Organism Visible BHV: Burrow Hole Visible

* Contact Laboratory Coordinator if Dissoved Oxygen level is < 2.5 mg/L or Temperature is out of range.

*If DO declines by more than 1.0 mg/L since previous measurement, then measure daily.

GLEC
Great Lakes Environmental Center

20-Day *Chironomous dilutus* WEIGHT DATA

Sample ID:_____ Test Species: *C. dilutus* _____ Type/Model of Drying Oven: Blue M

Project Name:_ Weigh Date:_____

Technician's Initials:_____ Test Date:_____

Oven Temperature: 60 °C
Drying Duration (Hours): ~ 24 hrs
Date/Time in: _____
Date/Time out: _____

Dessicator
Date/Time in: _____
Date/Time out: _____

Oven Temperature: 550 °C
Drying Duration (Hours): 2 hrs
Date/Time in:
Date/Time out:

Dessicator
Date/Time in: _____
Date/Time out: _____

QC'd by:_____

Sample ID	Replicate Number	**A** Number of Organisms at Test Initiation	**B** Dry Weight of Pan and Organisms (g)	**C** Ashed Weight of Pan and Organisms (g)	**B-C** Total Ash-Free Dry Weight (g)	**D** Number of Organisms Weighed	**B-C/D** Average Ash-Free Dry Weight (mg)	**B-C/A** Biomass Weight (mg)
Sample ID:	1							
	2							
GLEC Number:	3							
	4							
	5							
	6							
	7							
	8							
						AVERAGE:		

QC'd by:_____ Page ___of

Sample ID:

Chironomus dilutus Long Term Survival, Growth, and Emergence Whole Sediment Toxicity Test

GLC#:

Sample ID:

Test Species: *Chironomus dilutus*

Date Addition of Sediment:

Test Initiation Date:

Test Photoperiod: 16:8

Test System: 175mL Manual Delivery

Test Temperature: 23± 1°C

Test Organism Source/Age:

Test Termination Date:

Hatched Individual	Replicate _ (Hatch Date/Sex)	Replicate _ (Hatch Date/Sex)	Replicate _ (Hatch Date/Sex)	Replicate _ (Hatch Date/Sex)	Replicate _ (Hatch Date/Sex)	Replicate _ (Hatch Date/Sex)	Replicate _ (Hatch Date/Sex)	Replicate _ (Hatch Date/Sex)
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
Hatched Totals	_____	_____	_____	_____	_____	_____	_____	_____

20 Day Survival	Rep _____ - _____/12	Rep _____ - _____/12	Rep _____ - _____/12	Rep _____ - _____/12

Standard Operating Procedure for Static-Renewal Short-Term Chronic Toxicity Tests With Fathead Minnows (*Pimephales promelas*)

I/II. Scope/Purpose

1.1 This Standard Operating Procedure (SOP) describes the methodology for conducting short-term chronic static-renewal effluent toxicity tests with immature fathead minnows (*Pimephales promelas*).

1.2 The survival and weight data are used to estimate a no-observed-effect-concentration (NOEC) which is the highest concentration of the test material which results in no significant adverse effects on survival or fish weight when compared with the control data and/or IC_p values which are the concentrations of test material that cause a selected percentage reduction in young production.

III. References

3.1 All procedures in this SOP are based on procedures described in "Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms", Third Edition 1994, (EPA/600/4-91/002).

IV. Definitions

None.

V. Procedures

5.1 Test System

5.1.1 Test Organisms. Fathead minnow fry (*Pimephales promelas*) less than 24 hours old at test initiation, are used as test animals.

5.1.2 Food and Feeding. Test animals are fed a concentrated slurry of brine shrimp least two times daily at about a 6-hour interval (e.g. 1000 and 1600 hours). The nauplii should be rinsed with freshwater prior to use.

5.1.3 Test Chambers/Test Volume. Glass beakers (600 ml), containing at least 250 ml of test solution or dilution water (control) are used for all chronic fathead minnow toxicity tests.

5.1.4 Methods. Immature fathead minnows (<24-hours old at test initiation) are placed in test chambers and subjected to test conditions for 7 days. At least four beakers (10 organisms per beaker containing 250 ml of test solution) are used for each test concentration and control. The beakers are covered with a loosely fitting glass cover to minimize evaporation and keep out foreign debris. Survival is checked daily and dead fish are removed. Test solutions are changed daily. Fish weight is measured at the end of the test.

5.1.5 Test Materials. Test materials are stored at 4°C in the dark (unless otherwise specified), but allowed to gradually come to 25 ± 1°C before the test is initiated. Dilutions of the test material are made in volumetric flasks or graduated cylinders and then poured into the test beakers.

5.1.6 Dilution Water. Dilution water will be laboratory water unless otherwise specified in a study plan. If receiving water is specified as the diluent, a laboratory water control will be set up concurrently as a quality control measure.

5.1.7 Test Concentrations. The number of concentrations to be tested will be 5 and will be made with a dilution factor of either 0.5 (e.g., 100, 50, 25, 12.5 and 6.25 percent) or concentrations specified in the permit. The highest concentration to test may be determined by a screening test using order of magnitude dilutions of the effluent (e.g., 100, 10, and 1 percent) with 5 or 10 fish in 250 ml beakers containing 200 ml of solution for each concentration and control. The screening test solutions do not need to be duplicated but will aid in determining the test concentrations. For example, if all animals die in 100 percent effluent and no animals die in 10 percent, the following concentrations could be tested for the definitive test: 100, 50, 25, 12.5, and 6.25 percent effluent.

5.1.8 Controls. Controls will be set up and treated identically with regard to experimental conditions as the test chambers, except that no test material is added.

5.1.9 Replication. At least four test chambers are required for each experimental condition. Each of the test chambers contains 10 fish (a total of at least 40 fish per concentration). The number of replicate beakers per concentration and the number of fish per replicate may be less if specified in study specific plan.

5.1.10 Aeration. Aeration of test solutions is usually not performed during testing, except when the dissolved oxygen concentration of the test solutions is less than 4.0 mg/L of dissolved oxygen at 25°C. The water is then gently aerated by placing a glass gas dispersion tube or a glass pipette in each test chamber. The aeration rate should not exceed 100 bubbles/minute. The time when aeration is initiated must be recorded on the data form.

5.1.11 Light and Photoperiod. Fluorescent light bulbs (ambient laboratory illumination) with a 16-hour light:8-hour dark photoperiod automatically controlled are used with ambient laboratory levels of light intensity (e.g., 50-100 foot candles (fc)).

5.1.12 Temperature. Tests are conducted in a controlled environment chamber which maintains the water temperature in test chambers at 25 ± 1°C. A continuously operating recording thermometer provides a permanent record of the chamber temperature and is checked daily during the test period.

5.1.13 Water Quality Measurements. Specific conductance, hardness and alkalinity are measured in each new sample (100 percent effluent or receiving water) and in the control(s). Dissolved oxygen, pH, and temperature of new solutions are measured daily in one test chamber at the high, medium and low test concentrations, and in the control(s). Temperature, pH and dissolved oxygen should be measured daily on old solutions in one randomly selected chamber at the high, medium and low test concentrations, and in the control(s).

5.1.14 pH. If the pH of the test material is initially between 6.0 and 9.0, no adjustments are required. If not, the pH of the test material must be adjusted by using sodium hydroxide to raise the pH or by using hydrochloric or sulfuric acid to lower the pH. The pH of the test material must be measured and adjusted before beginning the test.

5.1.15 Toxicant/Effluent Renewal Frequency - Daily. Toxicant renewals should be performed within ± two hours of test start time.

5.1.16 Test Duration. 7 days (± 2 hours).

5.1.17 Experimental Endpoints - Survival, and growth (weight).

5.2 Day Before Test

5.2.1 Transfer tiles with fertilized eggs into a container with clean, vigorously aerated water 24 hours before the start of the test to ensure that only <24 hour old fathead minnows will be available for testing (note time of change in record book). If fish are purchased for the test verify the order.

5.3 Start of Test (Day 0)

5.3.1 Label test chambers with test concentration, test number and replicate letter or number.

5.3.2 Prepare test concentrations and add at least 250 ml of test solution to each test chamber.

5.3.3 Measure and record on data form the specific conductance, hardness and alkalinity of the effluent and control waters at test initiation.

5.3.4 Measure temperature, pH, and dissolved oxygen (DO) concentration in one test chamber at the high, medium and low test concentrations, and in the controls.

5.3.5 Randomly add fry (<24 hours old) to each test chamber (10 fry per chamber; at least 40 fry per concentration) following SOP on randomization of fish. Record the time the first fry is added. This is the start time of the test.

5.3.6 Randomly remove and preserve an equal number of fry as in each concentration (in groups of 10 fry) in 70 percent ethyl alcohol at test initiation for weighing at a later date.

5.3.7 Add a concentrated slurry of live brine shrimp (0.1 ml of slurry, 700-1000 brine shrimp) to each test chamber. The nauplii should be rinsed with freshwater prior to use. If the test is started early in the day, brine shrimp can be added to each test chamber again later in the day.

5.3.8 Check controlled environment chamber (or room) to ensure it is within specifications for temperature (25 ± 1°C), photoperiod (16 hr. light:8 hr. dark), and light intensity 50-100 foot candles (fc).

5.3.9 Place test chambers in controlled environment chamber (or room) and cover loosely with glass to minimize evaporation and keep out foreign particles.

5.4 Day 1 through Day 6

5.4.1 Add brine shrimp to each test chamber at least two times daily at about a 6-hour interval (e.g. 1000 and 1400 hr). The nauplii should be rinsed with freshwater prior to use.

5.4.2 Prepare new test solutions and bring the solutions up to test temperature (25 ± 1°C).

5.4.3 Record the time the test is checked.

5.4.4 Check condition (live/dead) of test animals and record on data form.

5.4.5 Remove dead fish.

5.4.6 Measure pH, DO, and temperature in one randomly selected chamber at the high, medium and low test concentrations, and in the control(s) in old (24 hours) solutions. Record on data form.

5.4.7 Siphon debris and old test solutions from each test chamber into a pan or bucket. After each test chamber is cleaned, examine pan or bucket to ensure no fish were siphoned. If any fish are found in pan or bucket, make a note on the data sheet and record as a technician error. Siphon old test solution to within 7 to 10 mm of the bottom.

5.4.8 Measure and record the specific conductance, hardness and alkalinity in each new sample (100 percent effluent or receiving water) and in the control(s).

5.4.9 Measure pH, DO, and temperature of new solutions in one test chamber at the high, medium and low test concentrations and in the control(s). Record on data form.

5.4.10 Add new test solutions to each test chamber by slowly pouring the solution down the edge of the beaker.

5.4.11 Place test chambers in controlled environment chamber (or room) and cover loosely with glass.

5.4.12 Dispose of old test solutions.

5.5 Day 7 - (Test Termination)

5.5.1 Do not feed fish.

5.5.2 Record the time the test is terminated.

5.5.3 Check condition (live/dead) of test animals and record on data form.

5.5.4 Remove dead fish.

5.5.5 Measure pH, DO, and temperature in one random chamber at the high, medium, and low test concentrations, and in the control(s) in old (24 hours) solutions. Record on data form.

5.5.6 Use a sufficient amount of ice to kill the fish in each test chamber then remove the fish and place in labeled aluminum weighing pans for immediate drying in an oven. If fish cannot be dried immediately, remove fish from each test chamber and place in labeled vials containing 70 percent ethyl alcohol for weighing at a later date (within two weeks of test termination).

5.5.7 Clean glassware and associated equipment and return to its proper location.

5.5.8 Dispose of old test solutions.

5.6 Fish Weighing Method

5.6.1 Label aluminum weighing pans.

5.6.2 Remove all fish from each test beaker or remove all fish from each vial. Fish from vials containing 70 percent ethanol must be rinsed with deionized or distilled water, and blotted dry. Place in labeled aluminum weigh pans.

5.6.3 Place weigh pans in a drying oven set at 100°C for a minimum of 2 hours. (Note: Maximum drying time is 24 hours.)

5.6.4 After oven-drying, remove weigh pans (or boats) and place in a dessicator until the fry are weighed to prevent the absorption of moisture from the air.

5.6.5 Weigh fish using an analytical balance to 0.01 mg.

5.6.6 Divide the total weight of dried fish per test chamber by the number of fish weighed to obtain an average weight per fish or by the total number initiated (10) to obtain a biomass weight.

5.6.7 Record all data on weight form.

VI. Quality Control

6.1 Use properly cleaned glassware and equipment.

6.2 Records will be kept as indicated in the SOP. These are reviewed by the study director and/or supervisory personnel.

6.3 Verify survival and growth in the controls for validity of test (≥ 80 percent survival and average weight of ≥ 0.250 mg/fish).

Originated by: Mailee W Garton Date 5/5/11
Mailee Garton

Approved by: Dennis JM McCauley Date 5/5/11
Laboratory Manager

This page intentionally left blank.

Standard Operating Procedure for Static-Renewal Short-Term Chronic Effluent Toxicity Tests with *Ceriodaphnia*

I/II. Scope/Purpose

1.1 This Standard Operating Procedure (SOP) describes the methodology for conducting short-term chronic static-renewal effluent toxicity tests with *Ceriodaphnia dubia*. During this test, *Ceriodaphnia* are continuously exposed for 7 days to selected concentrations of the effluent or test material diluted with either receiving water or laboratory water. *Ceriodaphnia* survival and reproduction are recorded daily for the duration of the test period. The solutions of test materials are renewed daily during the test period.

1.2 The survival and reproduction data are used to estimate a no-observed-effects concentration (NOEC) which is the highest concentration of the test material which results in no significantly adverse effects on survival or reproduction when compared with the control data and/or ICp values which are the concentrations of test material that cause a selected percentage reduction in young production.

III. References

"Short Term Methods for Estimating the Chronic Toxicity of Effluent and Receiving Waters to Freshwater Organisms." Third Edition, 1994, (EPA/600/4-90/002) and Fourth Edition, 2002, (EPA/821/R-02/013).

IV. Definitions

None.

V. Procedures

5.1 Test System

5.1.1 Test Organisms. Young *Ceriodapnia* used for testing must come from laboratory reared animals (4-14 days old) (see SOP on culturing *Ceriodaphnia dubia*). Parental organisms must be transferred into new culture media with food within 24 hours before starting a test.

5.1.2 Food and Feeding. Test animals are fed a yeast/trout food/Cerophyl (YTC) suspension (see SOP on YTC preparation) and *Selenastrum capricornutum* (See SOP on algae culture) daily during testing. Food must be added in conjunction with the test solutions initially and when test solutions are renewed at a rate of 0.1 ml of YTC and 8.625×10^6 algal cells per 15 ml of test solution.

5.1.3 Test Chambers/Test Volume. 30 ml-borosilicate glass beakers or plastic cups containing 15 ml of test solution or dilution water (control) are used.

5.1.4 Methods. Young *Ceriodapnia* (less than 24-hours-old at test initiation) are placed in test chambers (30 ml beakers or plastic cups) and subjected to test conditions for at least 6 or 7 days. Ten beakers (one organism per beaker containing 15 ml of test solution) are used for each test concentration and for the control. The beakers are covered with a loosely fitting glass cover to minimize evaporation and keep out foreign debris. *Ceriodapnia* are transferred under the water surface with a large-bore pipette into clean test beakers containing new test solutions and food daily during the test. Reproduction is monitored by counting the number of live and dead young, which are discarded each time the adults are transferred to new test solutions.

5.1.5 Test Materials. If effluents are to be tested they must be stored at 4°C in the dark until used. The temperature of the effluent must be adjusted to 25 ± 1°C before the test is initiated. Effluent and food are mixed together and then 15 ml is added to each test chamber. Dilutions of the test material are made in volumetric flasks or graduated cylinders. The test solutions are renewed daily during each test, which is best accomplished by placing the newly prepared test solutions in clean beakers with food and then transferring the adult *Ceriodapnia* into the new test solutions.

5.1.6 Dilution Water. Dilution water will be laboratory water (see SOP on Preparation of Reconstituted Water) unless otherwise specified in a study plan. If receiving water is specified as the diluent, a laboratory water control will be set up concurrently as a quality control measure.

5.1.7 Test Concentrations. The number of concentrations to be tested is 5 and made with a dilution factor of either 0.5 (e.g. 100, 50, 25, 12.5 and 6.25 percent test material) or 0.3 (e.g. 100, 30, 10, 3 and 1 percent test material) as specified by sponsor. The highest concentration to test may be determined by a screening test (24 to 48 hours) using order of magnitude dilutions of the test material (e.g., 100, 10, and 1 percent) with five *Ceriodaphnia* in 50 ml beakers containing 40 ml of test solution for each concentration and control. The screening test solutions do not need to be duplicated but will aid in determining the test concentrations. For example, if all animals die in 100 percent and no animals die in 10 percent, the following concentrations could be tested for the definitive test: 100, 50, 25, 12.5, and 6.25 percent test material.

5.1.8 Controls. Controls will be set up and treated identically with regard to experimental conditions as the test chambers, except that no test material is added.

5.1.9 Replication. Ten chambers, each containing 1 *Ceriodaphnia* (a total of 10 animals), are required for each test concentration.

5.1.10 Randomization. *Ceriodaphnia* are assigned using a block randomization procedure from the culture stock to the test beakers. A two-stage transfer procedure is utilized to transfer the offspring of one culture organism across the concentrations of the test board so that all the organisms are genetically identical across the concentrations. Beakers are then randomly placed in the environmental chamber (see SOP on randomization).

5.1.11 Aeration. Aeration is not used. If the dissolved oxygen concentration of the effluent and/or the dilution water is below 40 percent saturation at test temperature, aerate before preparing test solutions.

5.1.12 Light and Photoperiod. Fluorescent light bulbs (ambient laboratory illumination) with a 16-hour light:8-hour dark photoperiod automatically controlled. Ambient laboratory levels of light intensity may be used (e.g., 50-100 foot candles (fc)).

5.1.13 Temperature. Test are conducted in a controlled environment chamber which maintains the water temperature in test chambers at 25 $\pm$ 1°C. A continuously operating recording thermometer provides a permanent record of the chamber temperature and is checked daily during the test period.

5.1.14 Water Quality Measurements. Hardness, alkalinity and specific conductivity are measured as a minimum on each new effluent sample received (100 percent effluent) and on each batch of laboratory control water. Dissolved oxygen, pH and temperature will be measured, as a minimum, in the high, middle, and low test concentrations, and in the control, every 24 hours. The water quality measurements will be taken on composited samples of new and old solutions.

5.1.15 pH. If the pH of the test material is initially between 6.0 and 9.0, no adjustments are required. If not, the pH of the test material must be adjusted by using sodium hydroxide to raise the pH or by using hydrochloric acid to lower the pH. The pH of the test material must be measured and adjusted before beginning the chronic test.

5.1.16 Toxicant/Effluent Renewal Frequency. Daily. Toxicant renewals should be performed within ± 2 hours of the start time of the test.

5.1.17 Test Duration. The test duration is 7 days or until 60 percent of control organisms have had 3 broods (6-8 days).

5.1.18 Experimental Endpoints. Survival and total number of young per female.

5.2 Day Before Test

5.2.1 Transfer parent generation to new culture beakers containing food 24 hours before the start of the test to ensure that only <24 hour-old *Ceriodapnia* which have all hatched within an 8 hour period, will be available for testing (note time of change on acclimation form).

5.3 Start of Test (Day 0)

5.3.1 Label test chambers with name of test material (or GLC No.), test concentration, and replicate number for each test chamber (i.e., 1, 2, 3, ...10).

5.3.2 Prepare 150 ml of each test concentration in graduated cylinders or volumetric flasks.

5.3.3 Measure and record in data sheets or record book the hardness and alkalinity of the high concentration and control water at the beginning of the test.

5.3.4 Measure temperature, pH, specific conductivity, and dissolved oxygen concentration on composite samples before distribution to the beakers in the high, middle, and low test concentrations and for the control, as a minimum. Record on data form under NEW column.

5.3.5 Add 1.0 ml of YTC (yeast/trout chow/cerophyll) and 8.625×10^7 algal cells (375 µl of 2.3×10^8/cells/ml of algae) to 150 ml of test solution and mix thoroughly.

5.3.6 Add 15 ml of test solution to each test chamber for each concentration (N=10 beakers per concentration).

5.3.7 Using a block randomization procedure add one (1) immature *Ceriodaphnia* (less than 24-hours-old at start of test) to each test chamber (10 *Cerio-daphnia* per concentration). Record the time the first animal is added. This represents the starting time of the test.

5.3.8 Check controlled environment chamber (or room) to ensure it is within specifications for temperature (25 $\pm$ 1°C) photoperiod (16-hour light:8-hour dark), and light intensity (50-100 foot candles (fc)).

5.3.9 Randomize control and test concentrations into rows on a tray and then cover with glass.

5.3.10 Place test chambers in controlled environment chamber and cover loosely with glass plates to minimize evaporation and keep out foreign particles.

5.4 Renewal of Test

5.4.1 Label clean test chambers.

5.4.2 Prepare new test solutions.

5.4.3 Measure and record the hardness and alkalinity of each new effluent sample received and the control water.

5.4.4 Measure temperature, pH, specific conductivity, and dissolved oxygen on newly prepared composite sample before distribution to the beakers in the high, middle, and low test concentrations and the control, as a minimum. Record on data form under NEW column.

5.4.5 Add food to test solutions in the appropriate amount.

5.4.6 Add 15 ml of new test solution to clean test chambers.

5.4.7 Record the time the test is checked.

5.4.8 Check condition (live/dead) of test animals and record on data form.

5.4.9 Transfer live adults to clean beakers. Discard all dead animals. Note: *Ceriodaphnia* are extremely sensitive to mechanical stress; therefore transfer with care ensuring that the *Ceriodaphnia* are released below the surface of the water. Record on data form if *Ceriodaphnia* are injured during transfer.

5.4.10 If young are present, count all young (live and dead) per chamber. Discard young after counting. Record on data form.

5.4.11 Composite old test solutions from test beakers within a concentration to obtain a sufficient volume of "old" solution (24-hours-old) to measure temperature, pH, specific conductivity, and dissolved oxygen concentration for the high, middle, and low test concentrations, as a minimum, and for the control. Record on data form in the OLD column.

5.4.12 Replace test chambers in controlled environment chamber (or room) and cover loosely with glass plates.

5.4.13 Dispose of old test solutions in the prescribed manner.

5.4.14 Clean glassware and return to its proper location.

5.5 Test Termination (Day 7 or until 60 percent of control adults have 3 broods)

5.5.1 Record time the test is checked.

5.5.2 Check condition (live/dead) of test animals and record on data form.

5.5.3 Count all young (live/dead) per chamber and record on data form.

5.5.4 Composite old test solutions from all beakers within a concentration to obtain a sufficient volume of "old" solution to measure temperature, pH, specific conductivity, and dissolved oxygen concentrations in the low, middle, and high test concentrations, as a minimum, and the control. Record on data form in the OLD column.

5.5.5 Dispose of old test solutions in the prescribed manner.

VI. Quality Control

6.1 Clean glassware and associated equipment and return to its proper location.

6.2 Verify all data is recorded on data sheets. These are to be reviewed by study director or supervisory personnel.

6.3 Survival in laboratory water control must be ± 80 percent and reproduction in controls must average 15 or more young per surviving female.

6.4 60 percent of surviving females in the controls must produce 3 broods of young.

6.5 Monthy reference toxicitant testing is conducted following the same testing method described in this SOP using sodium chloride as the toxicant. Control charts generated from the reference toxicity testing results are provided with toxicity tests and are used to demonstrate the health of the organisms used for toxicity testing.

Originated by: Maile Garton Date 5/5/11
Maile Garton

Approved by: JM Conley Date 5/5/11
Laboratory Manager