BENTHIC MACROINVERTEBRATE MONITORING

HOG ISLAND

Quality Assurance Project Plan

EPA Grant Funding Source:
Grant #:

Project Coordinator: Joe Graham, Sediment Data and Monitoring Coordinator
Wisconsin Department of Natural Resources
Address

Principal Investigators: Wisconsin Department of Natural Resources
Lake Superior Research Institute

Prepared: May 2011
Revision #: 0
Prepared by: Tracey Ledder, WDNR

Approvals: **Date:**

_____ _____
Joseph Graham, Project Coordinator

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Donalea Dinsmore, WDNR Quality Assurance Coordinator

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Kurt Schmude, Lake Superior Research Institute

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Approvals: **Date:**

Joseph Graham, Project Coordinator — 6/13/2011

Donalea Dinsmore, WDNR Quality Assurance Coordinator — 6/27/2011

Kurt Schmude, Lake Superior Research Institute — 5/23/2011

Distribution List

Project Coordinator	Joe Graham, WDNR
Office of Great Lakes	Donalea Dinsmore, QC Coordinator
Lake Superior Research Institute	Kurt Schmude

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EXECUTIVE SUMMARY

Remediation of the Hog Island Inlet/Newton Creek in 2005 removed 44,340 cy of sediment from the inlet. This was the second project to utilize Great Lakes Legacy Act funds and the first remediation-to-restoration project in the St. Louis River Area of Concern. Validation sampling after dredging of PAH and lead contaminated sediments showed that the remediation goals had been met. Post-remediation monitoring in 2006 showed the beginnings of recovery of the benthic community.

This site requires further monitoring for benthic community recovery determination and for updated information on the site for removal from the Wisconsin impaired waters list. Results from 2011 benthic macroinvertebrate samplings will assist in the determination of this site's habitat restoration status, and that determination would contribute toward the removal of the impaired habitat BUI within the AOC. Sediment sampling in the isthmus area will be tied to PID screenings in order to investigate residual contaminant levels in that area as relates to habitat restoration and health and safety considerations for site workers.

SECTION ONE: PROJECT DESCRIPTION

1.1 PROJECT ORGANIZATION

The Wisconsin Department of Natural Resources, Sediment Data and Monitoring Coordinator, will oversee this project, and carry out sediment sampling, assisted by the St. Louis River AOC Coordinator. Sediment cores and benthic organism analyses will be carried out by Lake Superior Research Institute.

Project Manager – Joseph Graham, Sediment Data and Monitoring Coordinator, WDNR

Project Support – Tracey Ledder, St.Louis River Area of Concern Coordinator, WDNR

QA Manager – Donalea Dinsmore, Office of the Great Lakes, WDNR

Laboratory Manager, biology – Kurt Schmude, Lake Superior Research Institute

Laboratory Manager, chemistry – Ron Arneson, State Lab of Hygiene, WDNR Liaison

- Kate Grams, Qaulity Assurance, PACE

1.2 PROJECT BACKGROUND

This project will provide post-remediation monitoring of the benthic macroinvertebrate population for the Hog Island Inlet remediation carried out in 2005. Newton Creek and Hog Island Inlet were shown to contain elevated levels of polyaromatic hydrocarbons (PAHs), diesel range organics (DRO) and metals in the early 1990s. (Final Construction Documentation and Post-Remediation Monitoring Report, Hog Island Inlet Remedial Action, December, 2006, SEH). Several remediation steps have occurred over the last decade. Dredging in 2005 removed sediment from the Hog Island Inlet and lower section of Newton Creek to the goal of 2.6 mg/Kg TPAH to prevent negative affects to benthic organisms and fish.

1.2.1 Location and General Information

The project site is Hog Island Inlet, within the St. Louis River, northeast of Superior, Wisconsin, immediately west of the Superior inlet on Lake Superior. Douglas County is the land owner. Hog Island Inlet is bordered by Ogdensburg Pier, Hog Island, the Hog Island isthmus wetland, and the mainland shore. Hog Island is undeveloped. The Ogdensburg Pier was previously developed as a coal storage area and petroleum depot but is currently vacant. A series of railroad tracks lie along the southwest side of the Inlet. State Highway 2 runs parallel to the railroad tracks and mainland shore at higher elevations. Figure 1 shows the location of the Hog Island Inlet.

1.2.2 Topography

The Inlet is a sheltered bay wetland connected to Superior Bay by a 50-foot wide channel. The inlet is approximately 17-acres, and is separated from the adjacent Loon's Foot Landing, on the east, by a narrow isthmus. The post-remediation depths range from one to seven feet. Hog Island, rising approximately 15 feet above the water on the east side of the inlet, was created by navigation channel dredging disposal of sediment in the 1920s and 1930s. Newton Creek enters the Inlet from the west, winding 1.5 miles from the Murphy Oil refinery through forested wetlands and residential areas before entering the Inlet.

Surficial soils in the vicinity of the area consist of Ontonagon silty clay loam and Rudyard-Bergland clay soils. These are moderately well drained to poorly drained soils formed in clayey lacustrine deposits. Surficial soils in the vicinity of the site are underlain by a thick sequence of glacial till and offshore lacustrine soils belonging to the Miller Creek Formation.

1.2.3 Site History

The Hog Island Inlet is part of the St. Louis River Area of Concern and is identified in the Remedial Action Plan, Stage One (1992). Hog Island itself was created by deposition of navigation channel dredge materials in the 1920s and 1930s, the isthmus was most likely created at a later date. Newton Creek, a tributary to the Hog Island Inlet, was determined to be contaminated with petroleum products in the early 1990's. Newton Creek (WDNR, 1995) was subcategorized into 12 segments (A-L), with Segment A being the most upstream segment of the creek (downstream from the Murphy Oil impoundment area). Cleanup of Newton Creek focused on the impoundment area and Segments A through K, beginning in 1997.

An Ecological Risk Assessment and Human Health Risk Assessment were completed in September 2003, prior to remediation of Hog Island Inlet/Newton Creek Segment L. The Ecological Risk Assessment concluded that the ecological risk associated with contaminated sediments was high. Dry weight concentrations of TPAH showed good correlation to toxicity test results. The concentration threshold for TPAHs associated with no or lowest observed effects was in the 2 to 3 mg/kg range. The Human Health Risk Assessment concluded that non-carcinogenic hazards at Hog Island Inlet were within acceptable ranges for both adults and adolescents engaging in recreational activites. The carcinogenic risk associated with swimming was slightly elevated for both adults and adolescents, but within acceptable limits for wading, shore use and fish consumption.

Dredging removed 60,520 tons of contaminated sediment from the Inlet and Newton Creek segment L in 2005 to meet a site remediation goal of 2.6 mg/Kg TPAH.

1.3 PAST DATA COLLECTION ACTIVITIES

Several sampling events in Newton Creek and the Hog Island Inlet occurred prior to site remediation in 2005. These sampling events included surface water and sediment chemistry, sediment toxicity and macroinvertebrate population studies.

Sediments were dredged from the Inlet and lower segment of Newton Creek. Post-remediation confirmation sampling of sediments for PAH analyses was carried out in 2005. The results showed that the target TPAH concentration goal of 2.6 mg/Kg was met. The most recent site sampling in June, 2006, included sediment traps and Hess stream bottom sampling in Newton Creek (Segments A, B, D, F, G, and L) and sediment core samples at three previously sampled locations within Hog Island Inlet and the previous background location. The Inlet and Segment L toxicity studies indicated no significant reduction of survival to organisms exposed to post-remediation sediments, and benthic populations showed potentially increased diversity, indicating a positive step toward improved environmental quality (it was noted that one year may not have been enough time post-remediation to re-establish an adequate organic sediment bed habitat).

1.4 PROJECT OBJECTIVES AND TASKS

This site requires further monitoring for benthic community recovery determination and for updated information on the site for removal from the Wisconsin impaired waters list. Results from 2011 benthic macroinvertebrate samplings will assist in the determination of this site's habitat restoration status, and that determination would contribute toward the removal of the impaired habitat BUI and degradation of benthos BUI within the AOC.

The project will conduct sediment core sampling in 2011, repeating sites and methods used in previous years/studies (3 Hog Island Inlet stations (H-1, H-10, and H-30) and 1 reference station (WL-2, near Loons Foot Landing) to assess recovery/re-colonization of benthic macroinvertebrates. Five replicates per station will be sampled for a total of 20 core samples. Sampling will be conducted by the end of September, and samples analyzed for benthic macroinvertebrates by the Lake Superior Research Institute (LSRI). The ideal date for sampling macroinvertebrates will be the month of June, in order to have a seasonal comparison with the previous samplings.

Contractors for Douglas County have noted sheens and petroleum odors, strong enough to induce headaches, while working on habitat restoration in the wetlands at the isthmus to Hog Island. This area was screened for visual contamination in 2002 and none was noted, there are only limited analytical results for the isthmus (Newton Creek System Sediment Contamination Site Characterization Report, 1995). This area was not remediated in 2005, or previously, because no free product was observed and sediment concentrations were below the remediation goal. In order to better understand the sheens and petroleum odor at the Site, a photoinonization detector (PID) will be utilized to screen sediment head space for volatiles. Sediment samples (n=4) across the "neck" of the isthmus to Hog Island will be analyzed for PAHs, DRO, TOC, and lead at locations chosen by higher PID responses. The DRO analysis, which detects diesel range organics including natural biodegradation organics, will include chromatograms. The lab will also supply a DRO standard chromatogram. The background sample, WL-2, will provide a background chromatogram for comparison.

1.5 QUALITY OBJECTIVES AND CRITERIA

Macroinvertebrate populations will be sampled at the same sites and in the same manner as sampled previously (Final Construction Documentation and Post-Remediation Monitoring Report, Hog Island Inlet Remedial Action). Taxa richess and total densities will be compared to the 2006 post-remediation sampling. The objective is to determine if the benthic macroinvertebrate population is recovering post-remediation, while habitat restoration is occurring.

The remediation goal of 2.6 mg/Kg TPAH will be the benchmark for PAH analyses at the isthmus. Laboratory analyses of DRO and PAHs will be utilized with the PID screening readings to approximate the residual contaminant levels on the isthmus. This information will be utilized by the WDNR, Douglas County and other site workers for safety and health considerations. The chromatograms will allow a qualitative estimate of the relative concentrations of DRO and natural biodegradation products that may be measured during the DRO analyses.

1.6 TRAINING

Staff and students involved in the macroinvertebrate sampling and identification will have undergone training as required by LSRI (see LSRI SOP FS/13).

Laboratory analysts at the State Lab of Hygiene will have undergone training as specified in their program.

Laboratory analysts at PACE Analytical Services will have undergone training as specified in PACE SOP S-GB-0-019-Rev.03.

PID screenings will be carried out by staff familiar with this method, following calibration and use procedures as described in the Instruction Manual (Thermo Environmental Instruments, OVM 580 B Instruction Manual, Franklin, MA, 1/96).

1.7 DOCUMENTS AND RECORDS

Documents and files related to this project will by kept with the St.Louis River Area of Concern files in the Superior office. Records include field data sheets and shipment documentation, laboratory records of sample handling, project records including contracts, and final report. Coring, sampling picking and sample identification lab sheets will be kept in the LSRI project file for five years.

Digital data (laboratory results and project files) will be added to the SWIMS database.

SECTION TWO: DATA GENERATION AND ACQUISITION

2.1 SAMPLING DESIGN

Sediment core samples for macroinvertebrate analyses will be taken in the Hog Island inlet at four sites previously sampled (or as close to as possible as allowed by site conditions). Sediment cores will be taken at a depth of 15cm, five replicates per sample site. Core samples will be taken at three sites within the Hog Island Inlet, and at one background site, used previously (referred to as Loon's Foot Landing).

Multiple sediment head space readings will be taken within the isthmus for volatile organic components screening by photoionization detector (PID). At least four transects will be run, from mainland to island, depending on water depth and accessibility. Periodic ambient air readings of the PID will be recorded as well. Four sediment samples, for analyses of DRO, PAH, TOC and lead, will be taken across the isthmus based on PID screening results. See Figure 2.

Sample Information SWIMS PROJECT CAP_1_2011

Sample ID	Station ID	Latitude	Longitude
WL-2	163297	46.702198	-92.035736
HI-1	163248	46.70327	-92.03884
HI-10	163258	46.70513	-92.04089
HI-30	163354	46.704853	-92.041374

2.2 SAMPLING METHODS

2.2.1 Sediment Samples Chemical Analyses

Sediment samples for laboratory analyses of PAH, TOC and lead will be taken with a stainless steel scoop. The vegetative matter will be removed and the top six inches of sediment will be homogenized and collected in enough volume for the required sample containers.

A dedicated plastic syringe (30 mL) will be used to collect sediment for DRO analysis in the same location. The syringe contents will be immediately placed in a tared 60-mL VOC vial supplied by PACE, three vials are recommended. The syringe will then be filled again, capped and shipped with the sediment vials for dry weight analysis.

2.2.2 Sediment Samples Macroinvertebrates

Sediment cores for macroinvertebrates will be taken by the Lake Superior Research Institute according to LSRI SOP FS/22.

2.2.3 Sample Containers, Preservation and Holding Times

Sample containers for laboratory analyses will be supplied by the State Lab of Hygiene and PACE Analytical Services. Sediment samples will be placed on ice, and shipped overnight to the appropriate Lab. A Chain-of-Custody form will accompany each cooler shipped.

Macroinvertebrate sample containers will consist of 1-liter plastic jars provided by LSRI. Sediment samples will be preserved in the field with 10% formalin solution and taken to the laboratory for picking and identification. Samples not picked within one week will be re-preserved with 70-80% ethanol solution.

2.2.4 Decontamination Procedures

Stainless steel sediment scoops will be used for sediment sample collection. Each scoop will be cleaned with tap water and Alconox, rinsed with distilled water, dried and wrapped in aluminum foil prior to site work. Each wrapped scoop will be placed into a plastic closeable bag and returned to the foil wrap and bag after its use on site. The scoops will be cleaned after site work is completed in the same manner in which they were pre-cleaned (detergent and water may be used initially, if necessary).

2.3 SAMPLING HANDLING AND CUSTODY

2.3.1 Field Handling Procedures

Sediment samples for macroinvertebrates will be labeled both inside and outside of each jar. Staff at LSRI will be assisted in the field by WDNR staff. Samples will be labeled according to historical location identification numbers (ie, HI-##). Field data will be recorded including core length, number of replicates, mesh size and preservative.

Four transects will be run between the mainland and the island for PID screening. Samples for PID screening will be placed in a numbered closeable plastic bag, sediment clumps will be carefully broken up by hand. Bag numbers will be recorded in relation to field transect location. Bags will be allowed to warm in the sun for 15 minutes (or alternatively, inside a warm vehicle) before the PID intake tube is introduced into the air space in the bag and the volatiles measured. After reading with the PID, readings will be recorded by number and site location, and the sediment will either be discarded on site or added to vials to be sent to the two laboratories for analyses. Ambient air will periodically be read with the PID as well and readings recorded.

Sample jars and vials for laboratory analyses will be labeled with appropriate identification information (site name, sample identification, date). Samples will be labeled for the site and year taken (ie, WL-11-1 through WL-11-4), as they are taken, utilizing PID readings to select locations. Sample containers will be placed in plastic bags and stored on ice immediately. Samples will be shipped by overnight courier. A separate Chain of Custody form will be filled out for each laboratory. See Appendix A.

2.3.2 Laboratory Handling Procedures

Macroinvertebrate samples will be picked in the LSRI laboratory according to LSRI SOP FS/14. Samples are rinsed with tap water, sieved, and specimens transferred into 3.7 mL scintillation vials. Vials are preserved with 70-80% denatured ethyl alcohol.

Samples will be logged in and analyzed according to each analytical laboratory's standard operating procedures. Samples should arrive at the labs on ice.

2.4 ANALYTICAL METHODS

2.4.1 Field Analytical Procedures

A photo-ionization detector unit will be procured from the Rhinelander office. The unit will be calibrated prior to field screening. Four transects, northwest to southeast, will be screened within the isthmus, from the Island to the mainland. Sediments will be scooped into a numbered Ziploc bag and manually disrupted inside the bag. The bag will be kept in a warm location for at least 15 minutes. The PID intake tube will be inserted into a small opening in the bag seal to read the headspace. Readings will be recorded on a field sheet. The calibration will be checked and recorded at the end of the day.

2.4.2 Laboratory Analytical Procedures

Macroinvertebrate samples will be identified according to LSRI SOP FS/13. Specimens will be identified to species when possible. Peer reviewed and published taxonomic keys are utilized. All student identifications are verified by the Senior Invertebrate Taxonomist. See LSRI SOPs in Appendix B.

Polycyclic-aromatic hydrocarbons (PAHs) will be analyzed according to Wisconsin State Lab of Hygiene ESS Org Method 1580. Total Organic Carbon (TOC) will be analyzed according to ESS Org Method 1560.

Lead will be analyzed according to EHD Metal Method 400.2 (Sample preparation EHD Metals Method 100.1, and digestion by EHD Metals Method 750.1).

Samples will be analyzed for DRO according to PACE SOP S-GB-0-019-Rev.03. The method is a solvent extraction, gas chromatography procedure. Detection and quantitation is based on FID detection response compared to a diesel component standard. See SOP in Appendix C.

2.5 QUALITY CONTROL

Quality control for the macroinvertebrate analyses will consist of sample picking and identification checks according to LSRI's SOPs.

Quality control in the State Lab of Hygiene will be carried out according to the laboratory Quality Assurance Management Plan and method SOPs.

PACE Analytical Services, Green Bay, is a Wisconsin-certified laboratory (Laboratory ID: 405132750). Quality control is handled according to laboratory Quality Assurance Management Plans and, for DRO analysis, SOP S-GB-0-019-Rev.03 (Wisconsin modified method for determination of diesel range organics).

2.6 EQUIPMENT MAINTENANCE

2.6.1 Field Instrument Preventative Maintenance

The PID is maintained according to an annual schedule. Daily maintenance includes battery charging, calibration and lamp window cleaning when necessary. These procedures are described in the Thermo Environmental Instruments OVM 580B Instruction Manual.

2.6.2 Laboratory Instrument Preventative Maintenance

Each analytical laboratory maintains equipment according to their Standard Operating Procedures and Quality Assurance Management Plans.

2.7 INSTRUMENT CALIBRATION

2.7.1 Field Instrument Calibration

The PID unit will be calibrated to background zero and a 100 isobutylene calibration gas at the beginning and end of each day, according to the unit's Instruction Manual (Thermo Environmental Instruments, OVM 580 B Instruction Manual, Franklin, MA, 1/96). Calibration responses will be recorded on field sheets that include PID data.

2.7.2 Laboratory Instrument Calibration

Each laboratory calibrates analytical instrumentation according to their Standard Operating Procedures.

2.8 INSPECTION OF SUPPLIES

Sample bottles and vials will be supplied by the appropriate analytical laboratory. Sample containers will be inspected for being pre-cleaned and intact, with the method appropriate preservative. If there is a problem with the received sample containers, the appropriate laboratory will be contacted immediately so that the correct number of the correct containers and preservative can be shipped.

2.9 NON-DIRECT MEASUREMENTS

No non-direct measurements are planned for this project.

2.10 DATA MANAGEMENT

Project documents and data will be added into the Wisconsin Department of Natural Resources SWIMS database.

SECTION THREE: ASSESSMENT AND OVERSIGHT

3.1 ASSESSMENTS AND RESPONSE ACTION

Field conditions at time of sampling may require adjustments in sampling method or locations. Reasons for changes will be documented and the project coordinator contacted for permission to continue. Weather conditions may require a delay in sampling (flood events) or site conditions may require adjustment of sampling locations.

Any sample handling or quality control check problems during chemical analyses may require that the analyses be re-run or the samples be re-taken. The Project Coordinator will make the necessary decisions.

3.2 REPORTS TO MANAGEMENT

Lake Superior Research Institute will provide a final report including sampling and sample identification information, along with a basic comparison of 2011 results to the previous site results.

Each analytical laboratory will provide a results package for the sediment analyses. The State Lab of Hygiene will provide data to SWIMS. We will request SWIMS compatible reporting from PACE in order to add that data to SWIMS. PACE will also provide chromatograms of DRO samples.

The project coordinator will report to the Office of Great Lakes on field work accomplished and analyses accomplished on a semi-annual schedule.

SECTION FOUR: DATA VALIDATION AND USE

4.1 DATA REVIEW

The macroinvertebrate data will undergo data review according to LSRI's SOPs.

Each analytical laboratory will review their analyses and flag any quality control items according to their procedures. This review will be included in the final data package.

4.2 VERIFICATION AND VALIDATION METHODS

Project personnel will compare resulting detection/quantification levels and quality control for the sediment sample results to the project criteria. It is the intent that detection level problems be identified to the Project Coordinator during the analysis so that corrective actions can be taken.

4.3 DATA USE

Benthic community data generated during this sampling event will be compared to existing pre- and post-remediation data. An increase in benthic community diversity, including species known to be less tolerant to organic pollution, will indicate that the site remediation has been successful.

The results of the sediment analysis and PID screening will be utilized for future site safety planning and habitat restoration decisions for the isthmus. This data and its interpretation will be shared with Douglas County staff working on the habitat restoration project.

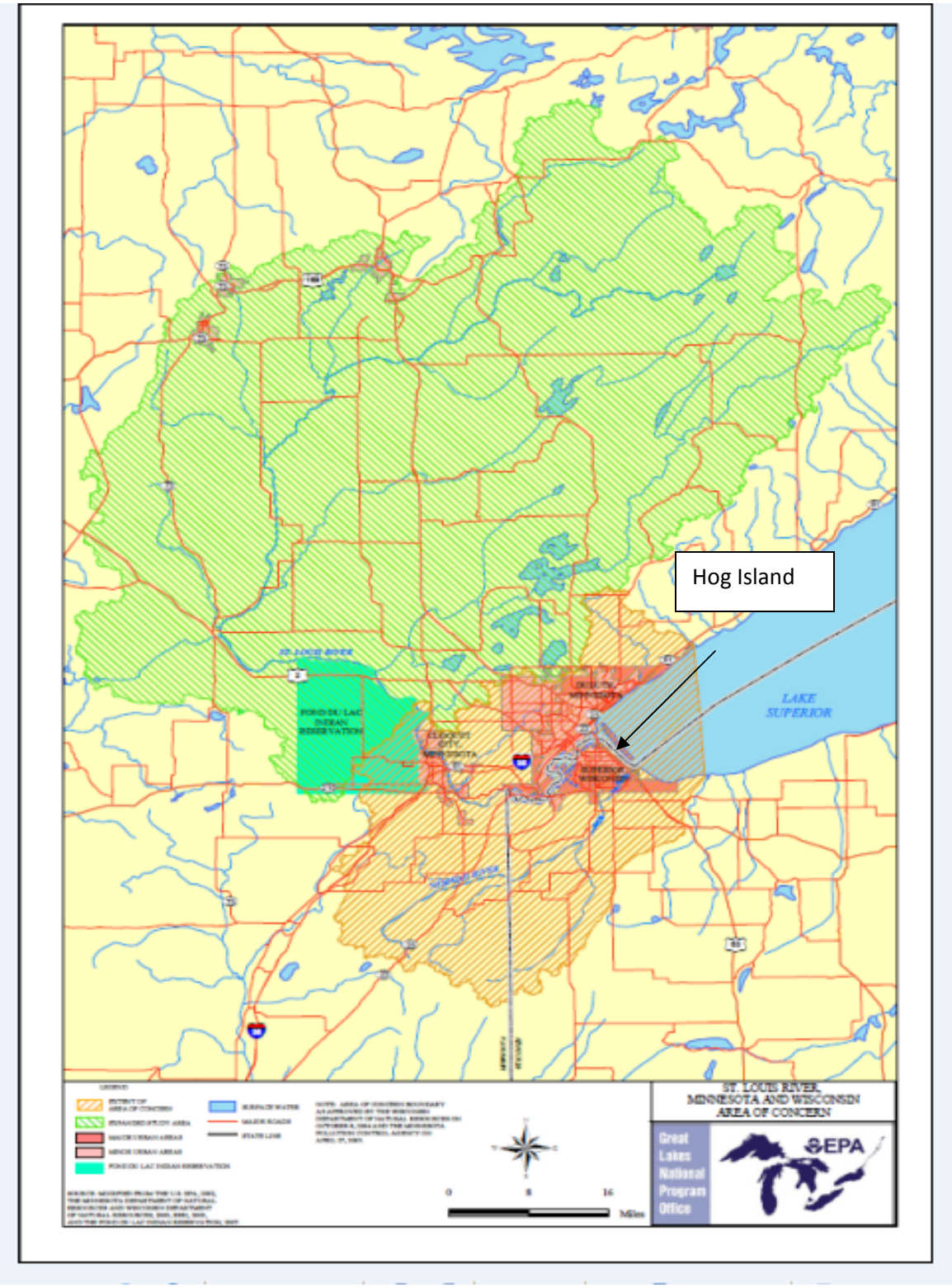
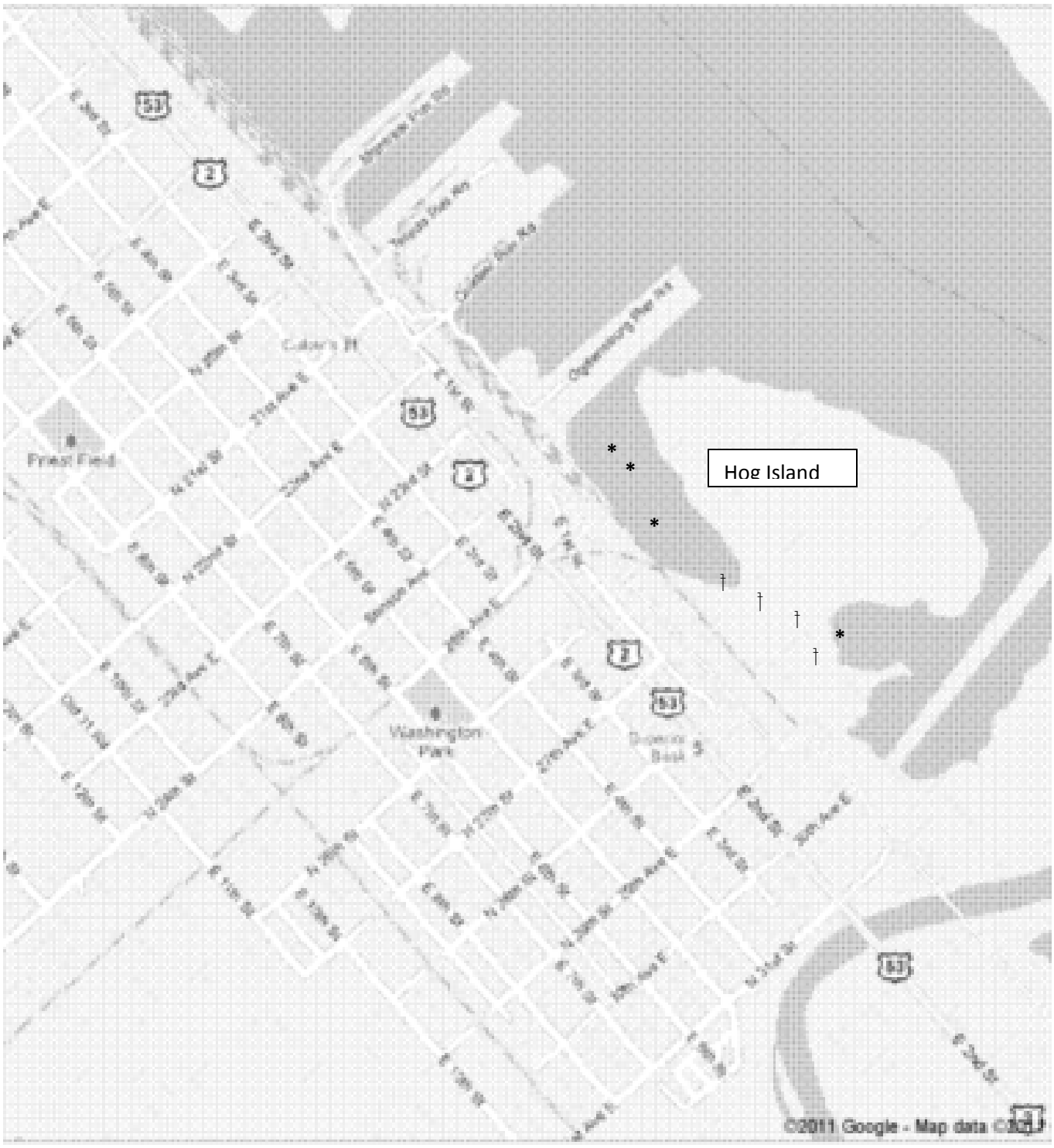


Figure 1 Location



***** Benthic Core Samples (approx. location)

†Sediment Samples dependent on PID readings

FIGURE 2 Sample Locations

Table 1 Laboratory Methods and Limits of Detection

State Lab of Hygiene

ESS ORG METHOD 1580 Polynuclear Aromatic Hydrocarbons in Soil and Sediment by GC/MS
SW846 Method 8270D

Parameter Code	DNR Parameter Description	Units	LOD	LOQ
61078	1-METHYLNAPHTHALENE	NG/G, WET	10	32
78305	2-METHYLNAPHTHALENE	NG/G, WET	5	16
99484	3,6-DIMETHYLNAPHTHALENE (WET WT)	NG/G, WET	10	32
78309	ACENAPHTHENE	NG/G, WET	10	32
78347	ACENAPHTHYLENE	NG/G, WET	5	16
78348	ANTHRACENE	NG/G, WET	5	16
78342	BENZO (A) ANTHRACENE	NG/G, WET	10	32
78343	BENZO (A) PYRENE	NG/G, WET	10	32
78344	BENZO (B) FLUORANTHENE	NG/G, WET	10	32
61075	BENZO (E) PYRENE	NG/G, WET	5	16
78349	BENZO (G H I) PERYLENE	NG/G, WET	10	32
78345	BENZO (K) FLUORANTHENE	NG/G, WET	10	32
78346	CHRYSENE	NG/G, WET	10	32
78352	DIBENZO (A H) ANTHRACENE	NG/G, WET	10	32
78323	FLUORANTHENE	NG/G, WET	5	16
78350	FLUORENE	NG/G, WET	5	16
78353	INDENO (1,2,3-C D) PYRENE	NG/G, WET	10	32
78331	NAPHTHALENE	NG/G, WET	10	32
78351	PHENANTHRENE	NG/G, WET	5	16
78354	PYRENE	NG/G, WET	5	16

ESS ORG METHOD 1560 Total Organic Carbon in Sediment by the Slurry Method
SW846 Method 9060

Parameter Code	DNR Parameter Description	Units	LOD	LOQ
81951	CARBON TOTAL ORGANIC	UG/G, DRY	2270	7220

EHD METALS METHOD 100.1, rev. 2, Preparation of Solid Samples for Metals Analysis, April, 2011.
EHD METALS METHOD 750.1, rev. 1, Digestion of Solid Samples for ICP, March, 2008.
EHD METALS METHOD 400.2, rev. 3, ICP, March, 2011.

Parameter Code	DNR Parameter Description	Units	LOD	LOQ
61852 or 1052	Lead	mg/kg, dry	1	3

PACE Analytical Services, Green Bay

SOP S-GB-O-019-Rev.03
WI Modified Method for Determination of DRO

Parameter Code	DNR Parameter Description	Units	DL	RL
	Diesel Range Organic	Mg/Kg, DRY	0.994	2

Table 2 **Quality Control Limits**

Laboratory	Parameter	Blank	Laboratory Control	Duplicate	Spike Recovery
State Lab	PAH	< LOD each parameter	Within 30%	Within LIMS QC limits	Within 3 SD of in-house data
	TOC	< LOD		Within LIMS QC limits	Within 30%
	Pb	< LOD	Within published limits for purchased standards	Within LIMS QC limits	Within 10% of expected
PACE	DRO	< 50 ug/L	Within 20% of expected value	Within 20%	70% - 120%

Procedure No: FS/22
Issue Date: April 25, 2011
Number of Pages: 04

STANDARD OPERATING PROCEDURE

COLLECTING BENTHIC MACROINVERTEBRATE SAMPLES USING A CORE SAMPLER

SOP Written by Kurt Schmude **Signature:**

Title: Senior Scientist

Date: 25 April 2011

Reviewed and Approved by Lana Fanberg **Signature:**

Title: Associate Research Specialist

Date: 04-25-2011

Cleared For Issue by Kelsey Prihoda **Signature:**

Title: Quality Assurance/Quality Control Manager

Date: 25 April 2011

DISTRIBUTION LIST:
LSRI Taxonomy Laboratory staff and students, any individual responsible for collection of benthic macroinvertebrate samples using a core sampler, LSRI QA staff, and LSRI Director.

RECORD OF REVISIONS:

No.	Date	Type	No.	Date	Type
1			7		
2			8		
3			9		
4			10		
5			11		
6			12		

Collecting Benthic Macroinvertebrate Samples Using a Core Sampler

INTRODUCTION

This Lake Superior Research Institute (LSRI) standard operating procedure (SOP) describes the routine method used to collect benthic macroinvertebrate samples using a core sampler. The coring device can be used when standing in a shallow aquatic habitat, or it can be used in deeper water from a boat. The maximum sample depth is limited by the length of the device and its extensions. The current device in use at LSRI has a length of six to seven feet.

Personal protective equipment (e.g. safety glasses/goggles, gloves, etc.) is required if core samples are collected from contaminated sediment. The type of gloves needed will be dependent upon the chemical contaminants present in the sediment. Consult the University of Wisconsin-Superior's Environmental Health and Safety Program Director in determining what type of protective equipment is needed for any project involving sampling from contaminated areas.

The amount or length of the sediment core collected is dependent on the project goals/objectives and on the habitat from where the samples are collected, and should be specified in the Quality Assurance Project Plan (QAPP) or other project planning documentation. Typically, the upper 15-cm of sediment is collected. In addition, the mesh-size of the sieve bucket is dependent on the project (as specified in the QAPP). At LSRI, a 250-μm mesh sieve bucket is typically used.

EQUIPMENT LIST

- 125-μm Mesh Sieve
- 1-Liter, Wide-Mouth Plastic Sample Jars
- 5-Gallon Bucket
- Filtered Ambient Site Water (filtered through the 125-μm mesh sieve)
- Funnel
- Large White Tray
- Large, Metal Spoon and Additional Smaller, Metal Spoons
- Pencil and Paper (for inside-jar sample labels)
- Permanent Marking Pen and Label Tape (for outside-jar sample labels)
- Piston-Coring Device (with the appropriate sample length clearly marked)
- Preservative (80% ethanol or 10% formalin solution)
- Project Log Book (waterproof paper preferred)
- Safety Gloves and Goggles/Glasses (if needed)
- Sieve Buckets (250-μm mesh or other appropriate mesh size); Total of 1 to 3
- Wash Bottles
- Wide Putty Knife

Reagents

80% Ethanol Preservative: Use 95% ethanol solution (this solution is equal to 100% alcohol) and dilute it to 80% with 17% (v/v) water and 3% (v/v) glycerol.

10% Formalin Preservative: Use 37% formaldehyde solution (this solution is equal to 100% formalin solution) and dilute to 10% with 90% (v/v) water. A buffered solution can be used by adding an acceptable base (e.g., calcium carbonate, sugar, etc.) to the water. ***Note: If the 10% formalin preservative is <u>not</u> buffered it must be replaced with 80% ethanol preservative within seven days of sample collection.***

SAMPLE HANDLING REQUIREMENTS

1. Store samples in labeled 1-L, wide-mouth, plastic sample jars.

2. Preserve samples in the field using either 80% (v/v) ethanol or 10% (v/v) formalin solution.

3. Replace the ethanol or formalin preservative in the samples with fresh 80% (v/v) ethanol preservative if the samples are not processed in the lab within a week. If the sample is preserved with buffered formalin, the sample can be stored for longer than one week before being processed. Consult the LSRI Taxonomy Laboratory supervisor to determine the maximum storage length (prior to processing) of samples preserved with buffered formalin.

4. Once re-preserved, the samples may be stored until they can be processed in a flammable storage cabinet.

PROCEDURE

1. Place the coring device gently onto the bottom substrate to avoid disturbance of the sediments.

2. Push the device into the bottom substrate until enough sediment is forced up into the tube to provide an effective "plug" that will hold the overlying sediment and water in the tube while it is raised to the surface.

    2.1. The proper amount of material needed to form the plug will depend on the type of sediments that are present. For example, clay will easily provide a "plug", while less dense materials (e.g., sand) will require a greater amount of sediment for a "plug". Trial and error will likely be necessary, which may be difficult in deep water. The piston portion of the coring device provides some suction to help hold the material within the tube, but the piston does not guarantee that the sediments will stay within the tube if there is no plug on the bottom of the tube.

    2.2. If collecting samples in shallow water, use a gloved hand (if needed for contaminated sediments) to cover the bottom of the coring device while it is lifted out of the water and into the sieve bucket.

3. As the end of the coring device nears the surface of the water, place a gloved hand (if needed for contaminated sediments) or the wide putty knife on the bottom of the core to

help ensure that the contents do not fall out of the tube. Place the end of the core inside a sieve bucket (250-μm mesh or other appropriate mesh size) as soon as the core leaves the water.

4. Typically, the upper 15-cm of sediment is collected; however, the amount/length of sediment is project- and habitat-dependent:

    4.1. If there is more sediment than the length/amount needed in the coring device for a particular sample, the bottom portion of the sample must be discarded.
    4.2. If less sediment than the length/amount needed has been collected, the sample must be discarded and another sample collected (following Steps 1-3 above). However, hardpan (i.e., clay-rich) sediments may be too difficult to obtain the proper amount of sediment, in which case less sediment may be collected.

5. Discard excess sediment by pushing slowly on the piston core and allowing the bottom portion of the sediment plug to extrude out the bottom.

6. Determine the proper length of sample to collect using the 15-cm mark (or other appropriate length, depending upon the project) on the coring device. Cut off the remaining portion of the sediment sample with the putty knife when the sample reaches the 15-cm mark (or other appropriate length marker).

7. Extrude the remaining sediment sample into the sieve bucket, along with the overlying water. Rinse out all sediments stuck on the inside walls of the coring device using wash bottles containing filtered ambient water from the site (i.e., filtered through a 125-μm mesh sieve into a clean, five-gallon bucket).

8. Sieve the sample in the sieve bucket until all fine materials pass through the 250-μm mesh sieve (or other appropriate mesh size, depending on the project objectives). Large debris may be kept or discarded according to the guidelines below:

    8.1. Clay balls may not entirely sieve out of the bucket. Carefully wash these balls by hand. Discard the clay balls if it can be determined with certainty that there are no organisms attached to, or encased in, the clay. Place the clay balls into the 1-L, wide-mouth, plastic sample jar if organisms are present on or in the balls.
    8.2. Discard large debris (sticks, leaves, miscellaneous materials) after they are thoroughly washed and inspected for macroinvertebrates. Alternatively, they may be placed in the sample jar for laboratory inspection.

9. Scoop out the majority of the fine sediments and debris remaining in the sieve bucket using a spoon and place into the sample jar. Rinse all remaining sediments and debris out of the sieve bucket and into a large white tray using a wash bottle filled with filtered ambient water from the site.

10. Pour the contents of the tray onto the 125-μm mesh sieve to drain the water from the sample. Collect the material to one side of the sieve and rinse the material into the

sample jar using preservative (i.e., 80% ethanol or 10% formalin solution) in a wash bottle and a funnel. Do not fill the sample jar more than halfway with sediment and debris. If necessary, use additional jars for the sample.

11. Add enough preservative to the sample jar(s) to approximately double the volume of the sediment and debris. Carefully invert the sample jar several times to thoroughly distribute the preservative.

12. **Label the sample with the appropriate information**, including: project name, locality, sample number, replicate number, date, and type of preservative. A label must be placed inside the jar (written using pencil) and on the outside of the jar (using label tape and permanent marker).

13. The coring device needs to be thoroughly rinsed with ambient water from the site (non-filtered) before it can be used to collect the next sample (ensure that the coring device is free from sediment and debris).

14. **All pertinent information for each field site sampled must be recorded in the project log book**; this includes sample collection date, name/initials of all individuals present, sampling location (record GPS coordinates if possible), number of replicate samples collected per site, length/amount of sediment core collected, mesh size of sieve bucket (i.e., 250-μm or other), type of preservative used, any observations made during sample collection, and any other information needed for data reconstruction.

Procedure No: FS/12
Issue Date: August 20, 1996
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STANDARD OPERATING PROCEDURE

SUBSAMPLING BENTHIC INVERTEBRATE SAMPLES IN THE LABORATORY

SOP Written by Kurt Schmude **Signature:**
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Date: 23 June 2010

Reviewed and Approved by Heidi Schaefer **Signature:**
Title: Research Specialist
Date: 29 June 2010

Cleared For Issue by Kelsey Prihoda **Signature:**
Title: Quality Assurance/Quality Control Manager
Date: 23 June 2010

DISTRIBUTION LIST:
LSRI Taxonomy Laboratory staff and students, quality assurance staff, and LSRI director.

RECORD OF REVISIONS:

No.	Date	Type	No.	Date	Type
1	03/06/2000	Changes in Introduction, Procedures #3, 5, and 14.	7		
2	06/21/2010	Formatted SOP. Added text to "Introduction". Added Figures 1 and 2. Added ¶2 and ¶3. Added "Quality Assurance/Quality Control" Section.	8		
3			9		
4			10		
5			11		
6			12		

SUBSAMPLING BENTHIC INVERTEBRATE SAMPLES IN THE LABORATORY

INTRODUCTION

This standard operating procedure (SOP) describes the subsampling method that has been developed by the Lake Superior Research Institute (LSRI) to expedite the processing of benthic invertebrate samples. The Benthic Invertebrate Subsampler (Figure 1) is modeled after the Folsom Plankton Splitter. Modifications include: a rectangular-shaped box with a V-shaped bottom and replacement of the collecting trays (replaced with spouts attached to the sampler) to facilitate removal of the subsample from the splitter. Tap water is added to the subsampler so that the sample is diluted to approximately a 50/50 mixture of water and sample. The sample is split in half, diluted again with tap water, and split in half again to obtain one-quarter of the sample. This process can be repeated again to obtain one-eighth of the sample, and so on. Debris and organisms collected in one of the subsamples are picked (according to LSRI/SOP/FS/14 – *Picking Benthic Invertebrates from Samples*), identified (according to LSRI/SOP/FS/13 – *Identification of Benthic Invertebrates*), and enumerated.

Figure 1. Photograph of LSRI's Benthic Invertebrate Subsampler, which is modeled after the Folsom Plankton Splitter. The Benthic Invertebrate Subsampler has a rectangular-shaped box with a V-shaped bottom and spouts attached to the sampler to facilitate removal of the subsample.

EQUIPMENT LIST

- 70% Denatured Ethyl Alcohol Solution
- Benthic Invertebrate Subsampler (Modified Folsom Plankton Splitter)
- Chemical Splash Goggles or Safety Glasses
- Clip and Knife
- Jars Containing Samples
- Lab Coat
- One-Quart Jar with Lid
- Permanent Marking Pen and Label Tape

- Plastic Splash Trays
- Safety Gloves (**Note:** Special gloves may be required when working with high-hazard materials. Consult the UW-S Environmental Health and Safety Director prior to starting.)
- Two-Gallon Bucket
- U.S. Standard Sieve, Size is Project-Dependent
- Wash Bottles

PROCEDURE

1. A lab coat, safety glasses/chemical splash goggles, and gloves must be worn at all times.

2. Workers are advised to see their supervisor *immediately* if they have any questions during the subsampling procedure.

3. Follow proper waste disposal procedures while subsampling.

4. Remove one labeled sample jar from the Flammable Storage Cabinet to a laboratory fume hood.

5. While under the fume hood, carefully pour the sample through a U.S. Standard sieve (deemed the appropriate size for a project) and into a dishpan or bucket.

   5.1. Cover the sample preservative that is collected in the dishpan/bucket and properly label it with the name of the preservative, concentration, date, initials, and any hazard communication information (e.g., "Danger – Flammable").

6. Rinse the sample jar thoroughly with tap water, and pour through the sieve. Rinse the debris and organisms that have been collected in the sieve with tap water (about two liters) to remove any remaining preservative solution. Gather the sample toward one side of the sieve, and rinse it into the benthic invertebrate subsampler with a wash bottle. ***Ensure that all debris and organisms have been removed from the sieve and are in the subsampler.***

7. Evenly mix the sample by gently rocking the subsampler back and forth for one-half to one minute, depending on the amount of debris that needs to be homogenized.

8. Split the homogenized sample into two equal halves/subsamples by pushing the dividing plate into place.

9. Place a three-gallon bucket under the discharge area of the subsampler. Tilt the subsampler downward and discharge one-half of the sample into the bucket.

10. Cut any organic debris that is caught on the edge of the dividing plate with a knife (half of the debris will be discharged in the bucket). Divide in half any sand that remains attached to the bottom of the subsampler by rinsing it with a gentle stream of tap water from a wash bottle. Rinse (with a wash bottle or hose) any debris or organisms remaining in the discharged half of the subsampler into the bucket.

11. Set aside the subsample in the three-gallon bucket to be split again if necessary.

12. Rotate the subsampler back to its original position. Rinse any debris or organisms that adhere to the inner walls of the subsampler and/or the dividing plate back into the subsampler using a wash bottle and tap water.

13. The remaining portion of the sample is split in half again (Figure 2), if necessary, following Steps 7-12 of this procedure; all subsamples that are discharged from the subsampler during Step 9 are kept separate if more splitting is required.

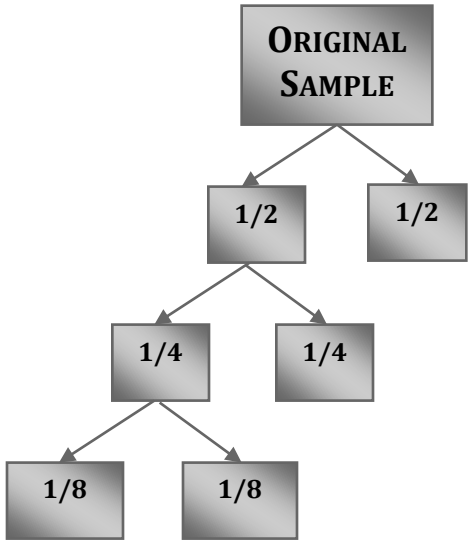

Figure 2. Diagram of the benthic invertebrate subsampling procedure. The original sample is split in half. This process is repeated if necessary. The subsample that is processed is randomly chosen during the subsampling procedure (i.e., by flipping a coin).

14. The subsample that will be processed (organisms picked) is randomly chosen.

15. The subsamples that will not be analyzed (one-half, three-quarters, seven-eighths, etc.) are rinsed out of the bucket with a wash bottle onto the appropriate U.S. Standard sieve. The subsample is then rinsed into properly labeled sample jars with a wash bottle containing denatured ethyl alcohol. All debris and organisms on the sieve must be placed into the jar. The sample is then preserved again. The initials of the worker, and the phrase "subsampled (enter date), identification of subsample (2 of 2, 3 of 4, 5 of 8, etc.)" must be written on the label. Also include the appropriate hazard communication information on the label (i.e., “Danger – Flammable”).

16. The original sample jar is stored in the appropriate storage cabinet.

17. All information on the sample's label is recorded in the data log book under the heading "Subsampling Log," or on the workbench data sheet.

18. The hooded work station and equipment must be cleaned before the next sample is processed.

Quality Assurance/Quality Control

Note: Quality assurance/quality control of benthic macroinvertebrate subsampling is project-specific; the following procedures are best-practices that should be implemented whenever possible.

1. Benthic invertebrate subsampling should only be conducted by personnel who have read and understood this SOP, who have been properly trained, and who have demonstrated competency in following this procedure. All procedures outlined in this SOP should be followed exactly; any deviations from this SOP should be approved (prior to subsampling activities) by a supervisor or project principal investigator.

2. Record data on pre-printed datasheets and/or in project-specific laboratory notebooks, following the documentation procedures outlined in the LSRI Quality Management Plan. Data storage time is project-specific, but typically does not exceed five years from the date the project is completed (i.e., final report is signed) or terminated.

3. A quality control (QC) check must be performed by qualified personnel who are experienced in the subsampling and analysis of benthic invertebrate samples. A QC check must be performed on the first sample that is subsampled for a given study in order to determine if there are any inherent, unusual circumstances that may contribute to subsampling bias (e.g., large size of organisms, heavy weight of organisms, etc.). If the first QC check meets the acceptance criteria, then a QC check should be conducted on 10% (1 out of 10, randomly selected) of the samples thereafter. All QC checks must be performed immediately following subsampling.

4. The QC check must consist of the processing of two randomly selected duplicate subsamples (e.g., randomly selected during the subsampling process by flipping a coin). Relative percent difference (RPD) between the two duplicate subsamples is quantified using the total number of organisms in each separately contained subsample. The RPD of the two duplicate subsamples is calculated using the following equation:

$$\text{RPD} = \left(\frac{|x_1 - x_2|}{\frac{x_1 + x_2}{2}} \right) * \ 100\ \%$$

Where: x_1= total number of organisms picked in subsample and
x_2 = total number of organisms picked in duplicate subsample

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Issue Date: August 20, 1996
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STANDARD OPERATING PROCEDURE
IDENTIFICATION OF BENTHIC INVERTEBRATES

SOP Written by Kurt Schmude **Signature:**

Title: Senior Scientist

Date: 23 June 2010

Reviewed, Approved, and Cleared For Issue by
Kelsey Prihoda **Signature:**

Title: Quality Assurance/Quality Control Manager

Date: 29 June 2010

DISTRIBUTION LIST:
LSRI Taxonomy Laboratory staff, quality assurance staff, LSRI director, and any individual responsible for identification of benthic invertebrates.

RECORD OF REVISIONS:

No.	Date	Type	No.	Date	Type
1	04/08/2003	Added more references.	7		
2	05/21/2010	Formatted SOP. Added text to "Introduction". Added reference for SOP and updated taxonomic references. Added "Syracuse Watch Glass" to "Equipment List". Added ¶1, ¶3, ¶5-¶7, ¶11, and ¶12. Added "Quality Assurance and Quality Control" section.	8		
3			9		
4			10		
5			11		
6			12		

IDENTIFICATION OF BENTHIC INVERTEBRATES

INTRODUCTION

This standard operating procedure (SOP) describes the method used for identification and enumeration of benthic invertebrates from samples received by the Lake Superior Research Institute's (LSRI) Taxonomy Laboratory. Samples are collected and then transported to LSRI for processing, subsampling, and/or picking (extraction). The following LSRI SOPs may be used to prepare samples for identification: LSRI/SOP/FS/16 – *Processing Hester Dendy Samples*, LSRI/SOP/FS/12 – *Subsampling Benthic Invertebrate Samples in the Laboratory*, and/or LSRI/SOP/FS/14 – *Picking Benthic Invertebrates from Samples*.
Benthic invertebrates are identified to the lowest taxonomic level possible based on current literature, or they are identified to the taxonomic level required by the project. Genus/species identification provides more accurate ecological and environmental information, but family-level identification provides a higher degree of precision among samples and taxonomists, requires less expertise to perform, and accelerates assessment results. Regardless of the taxonomic level of identification, only those taxonomic keys that are peer-reviewed and available publically (i.e., published) should be used (Barbour et al., 1999).

REFERENCES

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

REFERENCES: TAXONOMIC LITERATURE

Note: Numerous, additional taxonomic keys/publications, especially for the Chironomidae, are routinely consulted.

Epler, J.H. 2001. Identification Manual for the Larval Chironomidae (Diptera) of North and South Carolina. North Carolina Department of Environmental and Natural Resources.

Hilsenhoff, W.L. 1995. Aquatic Insects of Wisconsin. Keys to Wisconsin Genera and Notes on Biology, Distribution, and Species. Publication of the Natural History Council, University of Wisconsin-Madison, No. 3.

Kathman, R.D. and R.O. Brinkhurst. 1998. Guide to the Freshwater Oligochaetes of North America. Privately Published. Aquatic Resources Center, College Grove, TN.

Klemm, D.J. 1985. A Guide to the Freshwater Annelida (Polychaeta, Naidid, Tubificid, Oligochaeta, and Hirudinea) of North America. Kendall/Hunt Publishing Co., Dubuque, Iowa.

Merritt, R.W., K.W. Cummins, and M.B. Berg (Eds.). 2008. An Introduction to the Aquatic Insects of North America. 4th Edition. Kendall/Hunt Publishing Co., Dubuque, Iowa.

Pennak, R.W. 1989. Freshwater Invertebrates of the United States. Protozoa to Mollusca. 3rd Edition. John Wiley & Sons, Inc., New York. xvi + 628 pp.

Smith, D.G. 2001. Pennak's Freshwater Invertebrates of the United States. Porifera to Crustacea. 4th Edition. John Wiley & Sons, New York. x + 638 pp.

Thorp, J.H. and A.P. Covich. 2001. Ecology and Classification of North American Freshwater Invertebrates. 2nd Edition. Academic Press, Inc., New York. xvi + 1056 pp.

Wetzel, M.J., S.V. Fend, K.A. Coates, R.D. Kathman, and S.R. Gelder. 2006. Taxonomy, Systematics, and Ecology of the Aquatic Oligochaeta and Branchiobdellidae (Annelida, Clitellata) of North America, with Emphasis of the Fauna Occurring in Florida. A Workbook. 10 September 2006. vi + 269 pp + color plates.

Wiederholm, T. (Ed.). 1983. Chironomidae of the Holarctic region. Keys and Diagnoses. Three volume series. Entomologica Scandinavica Supplements.

EQUIPMENT LIST

- Data Sheets
- Dissecting and Compound Microscopes
- Empty Scintillation Vials
- Fine-Tipped Forceps
- Permanent Marking Pen and Label Tape
- Syracuse Watch Glass (see Figure 1)
- Tally Counter
- Taxonomic References
- Vials Containing Samples

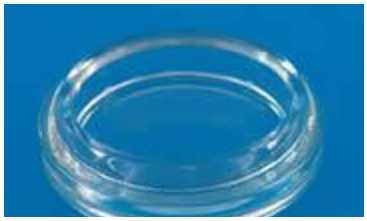

Figure 1. Syracuse watch glass for taxonomic identification of benthic invertebrates. Accessed from: http://www.emsdiasum.com/microscopy/products/grids/images2/71570.jpg, March 2010.

PROCEDURE

1. This procedure must only be conducted by taxonomists who have the appropriate training and experience in the identification of freshwater benthic invertebrates. Identifications are made by the senior taxonomist, trained biologist(s), or trained biology student(s). All identifications made by students and a proportion made by the biologists are verified by the senior taxonomist for accuracy.

2. Remove the appropriate benthic invertebrate samples (preserved in 70-80% denatured ethyl alcohol in labeled vials) from the Flammable Storage Cabinet and bring to a laboratory work station.

3. Record the sample label information on the project-specific data sheet.

4. Benthic invertebrates are identified and enumerated separately by taxonomic group while viewing through a compound microscope (e.g., Oligochaeta or larvae of Chironomidae), or dissecting microscope (e.g., all other invertebrates) using fine-tipped forceps. Only one

sample should be opened and processed at a single work station at a time; this will avoid mixing specimens among samples.

5. Pour the specimens from the vial into a Syracuse watch glass. Rinse the vial into the watch glass using 70-80% ethanol in a wash bottle. Add enough ethanol to the watch glass to cover the specimens.

6. Examine the vial label, vial, and its lid under a compound microscope for attached specimens.

7. Examine the sample under the compound or dissecting microscope and use taxonomic keys and other supportive taxonomic literature to identify the specimens.

8. Taxonomic identification level depends on the specimen. Benthic invertebrates are identified to the following taxonomic levels (unless otherwise specified by project requirements):

    8.1. Oligochaeta are identified to lowest taxonomic level possible, usually species. All other specimens are identified as pieces (without heads), immature tubificids (without chaetae), immature tubificids without hair chaetae, or immature tubificids with hair chaetae.

    8.2. Larvae and pupae of Chironomidae are identified to subfamily or tribe (very immature or damaged specimens), genus, species group, or species.

    8.3. Other macroinvertebrates are identified to the following taxonomic levels: insects to genus or species; Mollusca to family, genus, or species; Crustacea to genus or species; Hirudinea to genus or species; Nematoda to phylum; and Cnidaria to genus.

9. Place each taxon into separate 3.7-mL scintillation vials, or place all specimens from one sample into a single vial, depending on the objectives of the study. Vials are filled (one-half to three-quarters full) with 70%-80% ethyl alcohol for preservation.

10. Enumerate specimens as they are identified by manually marking on a data sheet or by using a counter.

11. Immediately record the following information on a project-specific datasheet: family, genus, or species; counts of larvae, pupae, and adults as appropriate for the taxonomic group; and any comments. Store the completed datasheets in a project-specific, three-ring binder.

12. Create a label for each vial that includes: sample identification code, collection date, taxon, number of individuals, initials of individual responsible for identification, date of identification, and any hazard communication information (e.g., “Danger – Flammable”).

13. Sample vials are stored together in a Flammable Storage Cabinet.

Quality Assurance/Quality Control

1. Identification and enumeration of invertebrates will be the responsibility of LSRI's Senior Invertebrate Taxonomist, Dr. Kurt Schmude.

2. All identifications made by students and a proportion made by the biologists are verified by the senior taxonomist for accuracy

3. All identifications will be based on current taxonomic literature.

4. Confirmation by outside expert taxonomists will be obtained if deemed necessary.

5. All invertebrates will be housed and maintained at LSRI upon completion of the project, or returned to the granting agency if required.

Procedure No: FS/14
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STANDARD OPERATING PROCEDURE
PICKING BENTHIC INVERTEBRATES FROM SAMPLES

SOP Written by Kurt Schmude **Signature:**
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Date: 23 June 2010

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Date: 29 June 2010

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Title: Quality Assurance/Quality Control Manager
Date: 23 June 2010

DISTRIBUTION LIST:
LSRI Taxonomy Laboratory staff and students, quality assurance staff, LSRI director, and any individual responsible for picking invertebrates from processed field samples.

RECORD OF REVISIONS:

No.	Date	Type	No.	Date	Type
1	07/19/1999	Not recorded.	7		
2	04/09/2003	Reformatted SOP.	8		
3	03/17/2010	Reformatted SOP. Added materials to the "Equipment List". Added ¶2, ¶3, ¶7, ¶11, and ¶20. Added "Quality Assurance and Quality Control" section.	9		
4			10		
5			11		
6			12		

PICKING BENTHIC INVERTEBRATES FROM SAMPLES

INTRODUCTION

This standard operating procedure (SOP) describes the method for extraction (picking) of benthic invertebrates from processed samples. Benthic invertebrate samples that are collected in the field are transported to the Taxonomy Laboratory at the Lake Superior Research Institute for processing (see *LSRI/SOP/FS/16* for details on processing Hester Dendy samples). Samples are rinsed through a sieve to remove the preservative, and debris and organisms retained by the sieve are transferred back into a sample jar. Small portions of the sample are placed into a gridded Petri dish for picking, and water is added to dilute the sample. Organisms are removed from each subsample using forceps while viewing through a dissecting microscope. The animals are separated into taxonomic groups (depending on the project requirements) and placed into vials containing ethyl alcohol.

EQUIPMENT LIST

- 3.7-mL Scintillation Vials
- 70%-80% Denatured Ethyl Alcohol Solution
- Chemical Splash Goggles or Safety Glasses
- Data Sheets
- Dishpans
- Dissecting Microscope
- Fine Forceps
- Lab Coat
- Permanent Marking Pen and Label Tape
- Petri Dish, Round and Gridded
- Plastic Spoon
- Processed Macroinvertebrate Samples
- Safety Gloves (**Note:** Special gloves may be required when working with high hazard materials. Consult the UW-S Environmental Health and Safety Director prior to starting.)
- U.S. Standard Sieve, Number 20 (for catching fine silt)
- U.S. Standard Sieve, Size is Project-Dependent

PROCEDURE

1. A lab coat must be worn at all times; chemical splash goggles and gloves must be worn during procedures 4-10, 13, and 18.

2. Workers are advised to see their supervisor *immediately* if there are any questions throughout the entire SOP.

3. Follow proper waste disposal procedures during the picking procedure.

4. Take a labeled sample jar from the Flammable Storage Cabinet where it is stored to a fume

hood. The sample jar should have been properly labeled by the individual(s) who collected the sample.

5. Decant excess preservative through a U.S. Standard Sieve (size deemed appropriate for a given project) into a separate container (e.g., jar or plastic container) to be saved.

    5.1. The saved preservative should be in a sealed or covered container and properly labeled with the name of the preservative, concentration, date saved, initials, and any hazard communication information (e.g., "Danger – Flammable").

6. Fill the sample jar with tap water and pour the contents onto a clean U.S. Standard Sieve (size is project-dependent). A dishpan must be underneath the sample at all times to prevent debris and organisms from being lost. Rinse the jar and lid thoroughly and gently with a water wash bottle and pour contents onto the sieve; no debris or organisms should be left in the jar or stuck to the lid.

7. Remove larger debris, such as sticks and rocks, from the sieve. Check the debris thoroughly for attached organisms. Rinse any attached organisms into the sieve, and dispose of the large debris in the trash if no attached organisms remain.

8. Partially fill a clean dishpan or plastic tray with tap water and gently rinse and sieve (i.e. gently circulate the contents of the sieve in water) the sample to eliminate the preservative and fine debris. The sample should be rinsed and sieved for a few seconds or up to 10-15 minutes depending on the amount of debris. Sieving can stop when the fine debris that passes through the sieve becomes negligible/water is clear. Pour the fine debris left in the dishpan/tray onto a U.S. Standard No. 120 Sieve to be collected and properly disposed in the trash. The rinse water may go down the drain.

9. Gather the sample toward one side of the sieve by using a plastic spoon and gently flowing water from a faucet. Use the spoon to gently scoop most of the sample off of the sieve and back into the rinsed sample jar. Carefully gather any remaining sample on the sieve with gently flowing water from a faucet, or wash bottle, and pour it into the same jar. Visually inspect the sieve for debris/organisms and place them into the jar; no debris or organisms should be left on the sieve. Cover the jar and take it to a work station. A work station within a fume hood (i.e., use large fume hood in Barstow 2) is required for samples that are deemed hazardous without proper ventilation. The UW-S Environmental Health & Safety Director will assist in making this determination.

10. Thoroughly clean the hooded work station and equipment so they are ready for the next sample.

11. Label an appropriate number of 3.7-mL scintillation vials with the sample identification code and the words "Danger-Flammable" (a hazard communication label may also be used), and fill with 70-80% denatured ethyl alcohol.

12. At the work station, place approximately one tablespoon of the sample into a gridded picking

tray (Petri dish). Add enough water to evenly distribute the "subsample" in the Petri dish, and view it under a minimum of 6x and a maximum of 12x magnification using a dissecting microscope on a light box.

13. Remove all macroinvertebrates from the one-tablespoon subsample using a fine forceps (macroinvertebrates may need to be counted as they are removed, this is project-dependent). Start at one end of the Petri dish and slowly move from grid to grid, searching through the debris, inside empty snail and bivalve shells and plant stems and leaves for small invertebrates. Place the organisms into labeled 3.7-mL scintillation vials containing 70-80% denatured ethyl alcohol. Fragments of organisms that allow for identification should be removed (e.g., head + first thoracic segment). Do not remove: insect exuviae, empty snail or bivalve (Mollusca) shells, eggs or egg masses, unless required by the project.

14. After the subsample has been picked, pour the debris in the Petri dish into a jar labeled exactly as the sample jar and include the phrase “Sorted Debris” on the label.

15. Repeat subsampling procedures 12-14 above until the entire sample has been picked, ensuring that all debris and organisms have been removed from the original sample jar. If the worker cannot complete the sample before the end of the day, or must leave for more than one hour, the sample jar, "Sorted Debris," and labeled vial(s) must be refrigerated and placed together. If a longer delay is expected and the sample will not be picked for several days, the sample must be re-preserved using the preservative saved in procedure 5, placed back into the Flammable Storage Cabinet, and re-processed as outlined above.

16. Place the labeled, 3.7-mL scintillation vials in a Flammable Storage Cabinet.

17. Enter on the project-specific data sheet the finishing date for the first picking and include the initials of the worker. Place the data sheet in the appropriate, project-specific binder.

18. Take the "Sorted Debris" container to a fume hood. Decant excess water through the appropriate U.S. Standard sieve (size is project dependent) and into the drain; return any debris on the sieve into the labeled “Sorted Debris” jar. Pour the preservative that was saved in procedure 5 into the jar, cover it, and place it into the appropriate Flammable Storage Cabinet. These jars must be returned to the granting agency or contractor for disposal if so stipulated in the contract. Otherwise, this material will be disposed as agreed upon with the UW-S Environmental Health & Safety Director.

19. For each sample record the following information in a project-specific laboratory notebook or an electronic log book under the heading “Picking Log”: initials of the primary picker, date of picking completion, the amount of time spent picking the sample, the number of total organisms picked (if the project requires counting), and the binder that the picking data sheet is stored in.

20. Thoroughly clean the work station and equipment so they are ready for the next sample.

Quality Assurance and Quality Control

Note: Quality assurance/quality control of benthic invertebrate picking is project-specific; the following procedures are best-practices that should be implemented whenever possible.

1. Benthic invertebrate picking should only be conducted by personnel who have read and understood this SOP, who have been properly trained, and who have demonstrated competency in following this procedure. All procedures outlined in this SOP should be followed exactly; any deviations from this SOP should be approved (prior to sample picking) by a supervisor or project principal investigator.

2. Record data on pre-printed datasheets and/or in project-specific laboratory notebooks, following the documentation procedures outlined in the LSRI Quality Management Plan. Data storage time is project-specific, but typically does not exceed five years from the date the project is completed (i.e., final report is signed) or terminated.

3. A quality control (QC) check must be performed by qualified personnel who are experienced in sorting and picking benthic invertebrate samples. All QC checks must be performed immediately following picking of the sample.

    3.1. A QC check should be conducted on 10% (1 out of 10, randomly selected) of an individual's picked samples for each project.
    3.2. The individual performing the QC check must go through the "Sorted Debris" container for the randomly chosen sample and count the number of benthic invertebrates found in the debris.
    3.3. Calculate the percent picking efficiency for each sample using the following calculation:

$$Percent\ Picking\ Efficiency = \left(\frac{A}{A+B}\right) \times 100\%$$

    Where: A = the number of organisms found by the primary picker
    B = the number of organisms missed by the primary picker and found during the QC check

    3.4. Ensure that a >90% picking efficiency is achieved. If an individual fails to achieve a >90% picking efficiency on a QC check, then QC checks should be performed on that individual's next five consecutive samples until a >90% efficiency is achieved.
    3.5. If an individual fails to meet the >90% picking efficiency on all five consecutive samples, corrective actions should be taken, such as re-training the individual.
    3.6. Allow a reduced accuracy (i.e., lower percent picking efficiency) in the following two situations (and based on the project objectives):

    - When a sample contains a low density of benthic invertebrates; low numbers of organisms can produce artificially high percentages of error. For example, if three organisms were found during the first pick of a sample, and two additional specimens were found during the QC check, then 40% of the organisms were missed during the first pick. However, only two specimens were missed overall.
    - When the percent picking efficiency does not have any effect on the interpretation of the data samples do not need to be repicked.

CHAIN-OF-CUSTODY / Analytical Request Document

The Chain-of-Custody is a LEGAL DOCUMENT. All relevant fields must be completed accurately.

Page: of

Section A **Required** Client Information:

Company:	
Address:	
Email To:	
Phone:	Fax:
Requested Due Date/TAT:	

Section B **Required** Project Information:

Report To:
Copy To:
Purchase Order No.:
Project Name:
Project Number:

Section C Invoice Information:

Attention:
Company Name:
Address:
Pace Quote Reference:
Pace Project Manager:
Pace Profile #:

REGULATORY AGENCY

☐ NPDES ☐ GROUND WATER ☐ DRINKING WATER

☐ UST ☐ RCRA ☐ OTHER_____

SITE LOCATION ☐ GA ☐ IL ☐ IN ☐ MI ☐ NC ☐ OH ☐ SC ☐ WI ☐ OTHER____

Filtered (Y/N)

Requested Analysis:

Section D **Required** Client Information

SAMPLE ID

One Character per box.
(A-Z, 0-9 / ,-)
Sample IDs MUST BE UNIQUE

Valid Matrix Codes

MATRIX	CODE
DRINKING WATER	DW
WATER	WT
WASTE WATER	WW
PRODUCT	P
SOIL/SOLID	SL
OIL	OL
WIPE	WP
AIR	AR
OTHER	OT
TISSUE	TS

ITEM #	SAMPLE ID	MATRIX CODE	SAMPLE TYPE G=GRAB C=COMP	COLLECTED COMPOSITE START DATE	COLLECTED COMPOSITE START TIME	COLLECTED COMPOSITE END/GRAB DATE	COLLECTED COMPOSITE END/GRAB TIME	SAMPLE TEMP AT COLLECTION	# OF CONTAINERS	Preservatives: Unpreserved	H_2SO_4	HNO_3	HCl	NaOH	$Na_2S_2O_3$	Methanol	Other	Residual Chlorine (Y/N)	Pace Project No. / Lab I.D.
1																			
2																			
3																			
4																			
5																			
6																			
7																			
8																			
9																			
10																			
11																			
12																			

Additional Comments:

RELINQUISHED BY / AFFILIATION	DATE	TIME	ACCEPTED BY / AFFILIATION	DATE	TIME

SAMPLE CONDITIONS

	Y/N	Y/N	Y/N	
	Y/N	Y/N	Y/N	
	Y/N	Y/N	Y/N	
	Y/N	Y/N	Y/N	
Temp in °C	Received on Ice	Custody Sealed Cooler	Samples Intact	

SAMPLER NAME AND SIGNATURE

PRINT Name of SAMPLER:

SIGNATURE of SAMPLER: DATE Signed (MM / DD / YY)

Pace Analytical Services, Inc.
1241Bellevue Street
Green Bay, WI 54302

Phone: 920 469 2436
Fax: 920 469 8827

STANDARD OPERATING PROCEDURE

WI MODIFIED METHOD FOR DETERMINATION OF DIESEL RANGE ORGANICS

Reference Methods:
Modified DRO Method for Determining Diesel Range Organics
– Wisconsin DNR – September 1995

SOP NUMBER:	S-GB-O-019-REV.03
EFFECTIVE DATE:	Date of Final Signature
SUPERSEDES:	S-GB-O-019-Rev.02

APPROVAL

Nils Melberg, Laboratory General Manager — Date: 4/05/2010

Kate Grams, Laboratory Quality Manager — Date: 03/25/2010

Glen Coder, Department Manager — Date: 03/25/2010

PERIODIC REVIEW
SIGNATURES BELOW INDICATE NO CHANGES HAVE BEEN MADE SINCE APPROVAL.

Signature	Title	Date

This is **COPY#** **15** Distributed on **4/29/11** by **JLB** and is ____ **CONTROLLED or** **X** **UNCONTROLLED**

Table of Contents

1. PURPOSE

1.1 The purpose of this Standard Operating Procedure (SOP) is to provide a consistent format to measure the concentration of diesel range organics in water and soil. This corresponds to a hydrocarbon range of C10 - C28 and a boiling point range between approximately 170^{O} C and 430^{O} C.

2. SCOPE AND APPLICATION

2.1 The policies and procedures contained in this SOP are applicable to all personnel involved in the planning, coordination, preparation, use and revision of SOPs.

2.2 The Practical Quantitation Limit (PQL) of this method for diesel range organics is approximately 2.0 mg/kg for soils and 50 ug/L for groundwater.

2.3 This method is based on a solvent extraction, Gas Chromatography (GC) procedure. This method should be used by, or under supervision of, analysts experienced in solvent extraction and the use of gas chromatographs. The analysts should be skilled in the interpretation of gas chromatograms and their use as a quantitative tool.

2.4 The method is designed to measure mid-range petroleum products such as diesel or fuel oil. Components greater than C28 present in products such as motor oils or lubricating oils are detectable under the conditions of the method. If, based on a review of the chromatograph the presence of these product types is suspected, additional analyses may be performed. These addition efforts are not contained within this method.

3. SUMMARY OF METHOD

3.1 This method provides gas chromatographic conditions for the detection of volatile petroleum fractions such as diesel, fuel oil #2, or kerosene. Samples are analyzed utilizing extraction to dissolve the organic constituents. The extract is dried, concentrated and injected into a capillary column gas chromatograph. The gas chromatograph is temperature programmed to facilitate separation of organic compounds. Detection and quantitation is based on FID detector response compared to a diesel component standard.

3.2 This method is suitable for the analysis of waters, soils, or wastes.

3.3 Soil core samples are collected in wide mouth vials with minimum handling to reduce loss of contaminants. Solvent preservation by extraction is performed in the lab.

3.4 This method is based in part on 1) USEPA SW-846: the 3rd edition of methods 8000 and 8100; 2) work by the EPA Total Petroleum Hydrocarbons Methods Committee; and 3) work by the Wisconsin Ad-Hoc Committee on LUST Program Analytical 4) Requirements and Wisconsin State Laboratory of Hygiene.

4. INTERFERENCES

4.1 Other organic compounds, including chlorinated hydrocarbons, phenols, and phthalate esters are measurable. As defined in the method, the DRO results include these compounds. Spills of neat products should be quantified by specific analysis for the product in question.

4.2 Washing all glassware with hot soapy water and then rinsing it with tap water and methylene chloride reduce method interferences. Reagent blanks must be analyzed with each batch or for every 20 samples to demonstrate that the samples are free from method interferences.

4.3 Contamination by carryover can occur whenever high-level and low-level samples are sequentially analyzed. Whenever an unusually concentrated sample is encountered, it should be followed by analysis of a solvent blank to check for cross-contamination.

5. SAFETY

5.1 All samples, standards, and reagents should be treated as hazardous. Safety glasses, gloves, and lab coats are to be worn. The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined. However, each chemical compound should be treated as a potential health hazard. Exposure to these chemicals must be reduced to the lowest possible level by a safe technique. Special care should be taken when handling the high concentration acids and oxidizing reagents used for sample digestion.

5.2 The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of any chemical. A reference file of Material Safety Data Sheets (MSDS) and a formal safety plan are made available to all personnel involved in chemical analysis and should be consulted prior to handling samples and standards.

5.3 Excess reagents, samples and method process wastes are characterized and disposed of in an acceptable manner. For further information on waste management consult the current version of S-GB-S-006, *Waste Handling and Management*, most current revision.

6. DEFINITIONS

6.1 Definitions can be found in Section 10 of the most recent version of the Pace Analytical Services, Inc. Quality Manual.

6.2 Diesel Range Organics (DRO): All chromatographic peaks eluting between n-decane (n-C10) and n-octacosane (n-C28). Quantitation is based on direct comparison of the area within this range to the total area of the 10 components in the Diesel Component Standard.

6.3 Diesel Component Standard: A ten-component blend of typical diesel compounds (Table1). This standard serves as a quantitation standard and a retention time window for diesel range organics.

6.4 Diesel Component Spike: A reagent water or method blank sample spiked with the Diesel Component Standard and run with 5% of all samples as a quality control check. At a minimum 1 Diesel Component Spike must be run.

7. SAMPLE COLLECTION, PRESERVATION AND HANDLING

7.1 Aqueous samples should be collected in a one liter amber bottle with a teflon-lined cap. The Teflon liner must contact the sample. Samples must be preserved with 5 mls of 50% HCl at the time of collection. Cool samples to 4^{o} C after collection. Extraction must be performed on waters within seven days of collection. Analysis must take place within 47 days of collection.

7.2 Soil can be collected using a 30 mL plastic syringe with the end sliced off. A sufficient number of vials (three is recommended) should be collected to provide for backup analyses in the event of breakage. One vial should be collected for dry weight determination. Samples should be collected in tared 60 mL VOC vials. Using a cut off syringe add a soil volume of 15-20 mLs (corresponding to about 25 g) to the vial or fill to mark on the vial. Excessive soil handling should be avoided. Be sure to clean all sediment from the vial threads. Samples can also be collected using the EnCore sampler. They are then transferred to 2 oz clear jars in the lab. Cool all samples to 4 C immediately after collection. Shipping time should be minimized. It is optimal for the lab to receive the samples within 4 days. 25 mLs of extraction solvent must be added to the soil sample within 10 days of sampling to insure preservation. The same solvent used for extraction must be used for calibration and analysis. For more details on soil sampling requirements see the "LUST Program QA Requirements." Analysis must take place within 47 days of collection. 35.4 grams is the maximum weight allowed for the WI. MOD. DRO method. If the DRO sample >35.4g the sample is subsampled and flagged.

7.3 Sample temperature must be determined upon receipt to the lab. If the sample was shipped in ice and solid ice is still present report the sample as "received on ice." Exact sample temperature need not be reported for samples received on ice. If, however, the sample was cooled using "blue ice" packs, or the ice used in shipping has melted then the temperature of a "temperature blank" must be reported. If the ice used to ship the sample has melted the temperature of the melt water may be substituted for a temperature blank. Note: If blue ice packs are used, precooling of samples to 4^{o}C with ice or by refrigeration is necessary.

7.4 The pH of all water samples must be determined unless the lab supplied sample vials containing acid for field preservation. The pH measurement may be performed on left over sample.

8. EQUIPMENT AND SUPPLIES

8.1 Gas chromatograph

8.1.1 Gas Chromatograph: Hewlett-Packard Series II equipped with a Hewlett-Packard 7673 Automatic Sampler.

8.1.2 Columns:

8.1.2.1 Column 1: 10 M x .53 mm DB-5, 0.32 micron film thickness; temperature limits - 60º C to 330º C, Restek

8.1.2.2 Other columns may be used - capillary columns are required. The column must be capable of resolving typical diesel components, and the solvent front from C10.

8.1.3 Detector: Flame ionization (FID).

8.2 Heated Water Bath used in a hood

8.3 Nitrogen evaporator with high purity nitrogen gas source.

8.4 Mettler BB600 Balance - A top-loading balance capable of weighing to the nearest 0.01 gram.

8.5 Elma Transsonic Digital Ultrasonic Bath.

8.6 Pressure Filter Apparatus.

8.7 VOC Vials and Bottles: Wide mouth 60 ml VOC vials with Teflon/silicone septa for soils. Amber 1 liter bottles with Teflon lined caps and preserved with 5.0 mL HCL for waters. Bottles are obtained from Eagle-Picher Environmental Services.

8.8 Separatory funnel – Labline or Chemisphere, 2000 ml with Teflon or glass stopcock.

8.9 Micro syringes - Hamilton or equivalent, 10 uL, 25 uL, 50 uL and 100 uL.

8.10 Disposable pipettes: Baxter Scientific Products.

8.11 Class A Volumetric(s): 2mL, 5mL, 10mL, 25mL, 50mL, 100mL.

8.12 TurboVap II Concentration Workstation.

8.13 Labline Extraction Mixer.

9. REAGENTS AND STANDARDS

9.1 Reagent Water: Barnstead Nanopure Infinity Ultra pure water system.

9.2 Solvents: Burdick and Jackson methylene chloride pesticide grade or equivalent.

9.3 Sodium Sulfate – Fisher S415-212 AR granular, anhydrous. Purify by heating at 400^{o} F for 4 hours in a shallow tray.

9.4 DRO free Ottawa sand – Fisher Brand S23-3. Baked at 400^{o} F for 4 hours in a shallow tray.

9.5 Stock Standards: Stock standard for the diesel components in methylene chloride Diesel Range Organics (20 mg/ml) obtained from Restek (Cat# 31064).

9.5.1 Transfer the stock standard solution into a Teflon-sealed screw-cap/crimp cap bottle. Store, with minimal headspace, at -10 C to -20 C and protect from light.

9.5.2 Standards must be replaced after 6 months or sooner if comparison with check standards indicates a problem.

9.5.3 ICV Stock Standard for the diesel components in hexane Diesel Range Organics (20 mg/ml) obtained from Supelco (Cat# DRH-001S-10X).

9.6 Diesel Component Standard: Using stock standard solutions, prepare Diesel Component Standard in a solvent, as needed. These standards should be stored with minimal headspace for volatiles and should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.

9.7 Calibration Standards: Prepare Calibration standards at a minimum of five concentration levels in solvent from the Diesel Component Standard (20 mg/ml from Restek). One of the concentration levels should be around the MDL. The remaining concentration levels should correspond to the working range of the GC.

10. CALIBRATION

10.1 Run the Diesel Component Standard at five concentration levels at and above the PQL and covering the linear range of the instrument. These levels are 50, 100, 250, 500, 1000 and 2000 ug/ml. Calibration standards are prepared by diluting the standard stock solution (20 mg/ml) from Restek with methylene chloride in 1, 2 and 10mL volumetric flasks. The stock standard is injected with a microsyringe below the surface of the methylene chloride in the volumetric and then the volumetric is diluted to the mark. The calibration standards are transferred to 2 mL vials with disposable pipettes. The vials are sealed with Teflon-lined aluminum crimp seals.

10.2 Inject each calibration standard. Tabulate peak area for the ten components against the mass injected. The results are used to prepare a calibration curve by linear regression.

10.3 Initial calibration accuracy must be evaluated before any samples are analyzed through the analysis of an Initial Calibration Verification standard (ICV) which includes all compounds of interest. The ICV should be at or near the midpoint of the calibration range, derived from a source independent of the calibration standard, (i.e., second source), and must quantitate within +/- 20%of the expected value. The ICV concentration is at 250 ug/l for the diesel fraction.

10.4 The working calibration curve must be verified on each working day by the injection of a calibration standard. If the response for the calibration standard varies from the predicted response by more than 20%, a new calibration curve must be prepared. The working range of the calibration curve is from 0-2000 ug/mL. The calibration check is at a concentration of 250 mg/L.

10.5 Reporting Limit Verification Standard (RLVS) – A standard prepared at the concentration of the Pace Reporting Limit. It is analyzed after the calibration and monthly thereafter, recovery 60-140% of true value. If outside the limits, reanalyze once. If still outside the limits, recalibrate.

11. PROCEDURE

11.1 Samples are analyzed by GC/FID. Waters are extracted using a separatory funnel technique. Soils are extracted in vial or jar. Details are given in section 12.4. After the extracts are concentrated, a volume is injected directly onto the GC.

11.2 Gas Chromatography

11.2.1 Set FID Detector to 300 C and injector to 300 C. MACH- 50 C for 1 minute, then 250 C/min to 320 C/min, hold for 5 min run time = 7 min

11.2.2 NOTE: Other conditions may be used.

11.3 Retention Time Window and Quantitation

11.3.1 Diesel Range Organics (DRO): All chromatographic peaks eluting between n-decane and n-octacosane. Quantitation is based on direct comparison of the area within this range to the total area of the 10 components in the Diesel Component Standard.

11.3.2 The retention time window is defined as beginning approximately .1 minutes before the retention time of decane and ending .1 minutes after the retention time of octacosane in the calibration run. PACE uses an HP-Chem software macro to automatically establish a DRO window and integrate fuel peaks within the window.

11.3.3 Quantify by summing all peak areas eluting between n-decane and n-octacosane. The baseline is kept constant over this range.

11.4 Sample preparation

11.4.1 Water extraction - Separatory Funnel

11.4.1.1 Transfer the sample to the 2-L separatory funnel. If the sample is in a 1-liter or smaller bottle, mark the water meniscus on the side of the sample bottle for later determination of the sample volume. If the sample is in a larger bottle, mark the water meniscus on the side of the sample bottle for sample volume determination. Pour the sample into a 2-liter separatory funnel. For blanks and quality control standards, pour 1 liter of reagent water into the separatory funnel.

11.4.1.2 Note the initial pH. If the sample bottle does not state that it is preserved, then check the pH using Whatman pH indicator paper.

11.4.1.3 Add 60 mLs solvent to the sample bottle to rinse the inner walls (same solvent used for the calibration standards). Transfer the solvent to the separatory funnel. Extract the sample by shaking it for three minutes with the Labline Extraction Mixer set at 30 cycles a minute.

11.4.1.4 Allow the layers to separate.

11.4.1.5 Drain the solvent layer through a funnel filled with Na2SO4 into a TurboVap concentrator tube.

11.4.1.6 Repeat the extraction once more using a 60 mL aliquot of solvent. Collect the solvent in the same tube described in 11.4.1.5.

11.4.1.7 Use the TurboVap II at 35-C in Sensor mode to concentrate sample to ~ 1ml. The pressure should be set at 11 psi. If the extract is highly colored or a precipitate forms during concentration, the final volume may be higher.

11.4.1.8 If the extract is highly colored or a precipitate forms during concentration, the final volume may be higher.

11.4.1.9 After the concentrator tube or TurboVap concentrator tube has cooled, rinse the sides of the bottom half the tube with a small amount of solvent. Bring the final volume to 1 mL. Transfer the extract to two 2ml vials with Pasteur pipettes.

11.4.1.10 Record the prep information for the extraction and concentration steps. The sample extract is ready for analysis in section 12.5.4.

11.4.2 Solvent Extraction for soil/sediment: This method is based on extracting the sediment/soil with solvent. An aliquot of the extract is concentrated and injected on the GC.

11.4.2.1 Weigh the tarred sample vials to determine the actual weight. See SOP: S-GB-O-018, *DRO Weight Determination* most current revision. If weight is not available, the sample is sub-sampled and weight is documented on the extraction log sheet.

11.4.2.2 Add 25 grams of dried Na2SO4.

11.4.2.3 Add 25 ml of methylene chloride within 10 days of sample date.

11.4.2.4 Well mix the sample by stirring with a steel spatula Sonicate for 20 minutes

11.4.2.5 Allow sediment to settle until a layer of solvent is apparent.

11.4.2.6 Decant the solvent into a filtering funnel pre-assembled with a glass wool plug and sodium sulfate. The sample extract is collected in a 60 mL disposable vial set underneath the funnel.

11.4.2.7 Repeat extraction once more as described in sections 12.4.2.3 and 12.4.2.4 except only 15 ml of CH_2Cl_2 is used. Pour all solvent and sample extract into the filter funnel, rinse the extraction jar with CH_2Cl_2 to remove any residual sample contents and pour into funnel. Rinse the sodium sulfate with CH_2Cl_2 and place the pressure filter apparatus on top of the funnel to pressure filter the sample into the 60 mL collection vessel. After all the sample extract has been pressure filtered, proceed to concentration.

11.4.2.8 Add 1-2 boiling chips to each 60 mL disposable vial containing sample extract and attach a three-ball micro-Snyder column.

11.4.2.9 Place the sample on the water bath with the bottom of the 60 mL vial partially immersed in water. The water bath temperature should range between 75° to 85° C. After heating to temperature, the balls in the micro-Snyder column should actively chatter but not flood with solvent.

11.4.2.10 Remove the 60 mL vial from the water bath when the extract volume has concentrated to approximately 500 uL. After the unit has cooled, rinse the Snyder column with CH_2Cl_2 collect solvent into the vial and remove the Snyder column. Transfer the sample aliquot into a 2mL autosampler vial marked and verified with 0.5, 1 and 1.5mL volume markings and adjust the final volume to 1mL.

11.4.2.11 Silica gel may be used as a clean up of the extract to remove any polar compounds that may be present. If silica gel is used on sample extracts it must also be used on all QC sample extracts.

11.4.3 Inject 1 uL of the concentrated extract onto the GC and proceed with the analysis.

11.4.4 If the sample extract exceeds the working range of the calibration curve, the sample must be run at a dilution that places the concentration of the extract in approximately the upper half of the calibration curve. The acceptable on column range is 900-2000 ppm or mg/L.

11.5 Calculations:

11.5.1 DRO Calculation: The concentration of Diesel Range organics in the sample is determined from a summation of peak area for all chromatographic peaks eluting between n-decane and n- octacosane, using the calibration curve. Refer to Section (Retention Time Windows and Quantitation). From linear regression of calibration standard GC response (R) against their known concentrations (C in ug/ml) derive the following linear equation:

$$C = mR + b$$

Using the slope (m) and the intercept (b) from this equation the concentration of the sample can be calculated from the following equations:

Water samples:

$$Cs = [(mRs + b) (Ve) (D)] / Vs$$

Soil samples:

$$Cs = [(mRs + b) (Ve) (D)] / W$$

Where:

Cs = Concentration of sample in ug/L for waters and mg/kg on a dry weight basis for soils

m = slope of the calibration curve

Rs = GC response of sample in the DRO retention time window

b = intercept of calibration curve

Ve = total volume of sample extract (after concentration) in ml

Vs = volume of water sample in liters

D = dilution factor of water or soil extract as diluted

W = total dry weight of soil sample in grams

11.5.2 PACE has automated data processing. The integrated results are automatically processed against the linear regression curve, transferred to EpicPro and then corrected for extraction volume, sample weight or sample volume and percent solids for soil samples.

Auto program:

Login to EpicPro through Windows.
Select Import Data.
Select Semivolatiles and then DRO.
Select the number of DRO instrument and directory.
Click on field names in row and then on Start Import.
Delete out any samples that need to be rerun.
Select Send To LIMS.
Select Create a New Worksheet.
Name the worksheet DRO (instrument #) (date run was started).
Close the door.
Select Enter Results and By Worksheet.
Chose the worksheet that was just named.
Select Calculate Results.
Close the door and exit EpicPro.
Another person in the Semivolatile section goes in to EpicPro to review the data and mark the samples approved.

11.5.3 The initial result off the GC, the total solids, dilution factor and the weight of the sample are sent to LIMS through the auto program. The final volume of 1.0 ml is preset in LIMS. The initial volume for waters is preset at 1.0 L. If a sample is not blown down to 1.0 ml or a water sample is not 1.0 liters, these parameters must be changed manually in LIMS.

11.5.4 The LIMS calculation.

IA=Initial amount - Value off the GC-transferred automatically in mg/L units.

D=Dilution factor - Transferred automatically.

V=Final volume - Set at 1.0 mL for waters, or 1mL for soils
(Must be changed manually if different.)

W=Weight - Soil weight in grams transferred automatically.
For waters initial volume is set at 1.0L.
(Must be changed manually if not 1.0L.)

S=SOL% - Total solids is transferred automatically for soils.
SOL% is set at 100% for waters.

DRO water result = (IA*D*V)/W
Results are in ug/L with reporting to 2.0 significant figures.

$$\frac{(mg/L)}{ml} \quad \frac{mg * ml}{L^2} \quad \frac{L}{1000ml} \quad \frac{1000ug}{mg} \quad \frac{ug}{L} = \text{Water units.}$$

DRO soil result = (IA*D*V)/(W*S)
Results are in mg/kg with reporting to 2.0 significant figures.

$$\frac{mg}{L} \quad \frac{1}{g} \quad \frac{ml}{1} \quad \frac{1L}{1000ml} \quad \frac{1000g}{kg} \quad \frac{mg}{kg} = \text{Soil units.}$$

11.5.5 Peak areas measured from blanks may not be subtracted from sample peak areas. Peaks from blank samples, which interfere in the window, and are above detection limits must be reported.

11.5.6 If there were significant peaks outside the chromatographic window, this fact must be reported. The analysis is extended at a minimum of 5 minutes after the last diesel component standard.

11.5.7 Comments on the chromatogram and/or other problems regarding the sample should be entered into the LIMS comment section and the reports.

12. QUALITY CONTROL

12.1 The analyst must make an initial demonstration of the ability to generate acceptable accuracy and precision with this method by successful analysis of the following:

12.1.1 Replicate Water spikes. Analysis of 5 replicates at a concentration of 250 ug/l with recoveries between 75%-115% of the known concentration. The RSD must be <20%.

12.1.2 Replicate Soil spikes. Analysis of 5 replicates at a concentration of 20 mg/kg with all recoveries between 70% and 130% and precision of all the replicates within 20%. Soil spikes should be prepared and analyzed as described in section 11.

12.2 With every batch of 20 samples or less the lab must analyze:

12.2.1 Duplicate Laboratory Spike - Water: The RPD must be <20%. The replicate spikes must be between 75%-115% of the known concentration. Water component spikes are prepared from an intermediate standard in which 3250 uL of the 20 mg/ml Diesel Component Standard (Restek) is injected into a 250.0 mL volumetric flask and brought up to volume with methylene chloride. 1000 uL of the intermediate standard is then injected into 1.0 L of organic free water and extracted using the method described in section 11.4.1. The final theoretical concentration of the water spikes is 250 ug/L.

12.2.2 Duplicate Soil Spike. The soil spike is prepared by spiking the Diesel component Standard into a sample of clean Ottawa sand. Soil spikes must be prepared at least 24 hours before extraction and analysis with a batch of samples and should be held at 4 C. 25 grams of Ottawa sand is injected with 250 uL of 2000 ug/mL DRO standard from Restek. 25 mL of methylene chloride is added to the sand and the sample is stored at 4 C. The spike is ready to be extracted with a batch of samples. The theoretical value of the spike extract is 20.0 mg/kg. The spike recovery must be between 70% and 120%. The RPD must be <20%.

12.3 Calibration Standard: The peak area must fall within 20% of the value predicted by the calibration curve. A 250 ug/mL standard is prepared by diluting 250 uL of 10 mg/mL DRO standard to 10 mL with methylene chloride in a 10 ml volumetric flask.

12.4 Solvent Blank - Methylene Chloride

12.5 Method Blank - Water: Barnstead purified water is processed through the method in the same manner as a sample. If the concentration exceeds 50 ug/L, the samples associated with batch must be rerun or flagged.

12.6 Method Blank - Soil: Sand is processed through the entire extraction procedure. If the concentration exceeds 2.0 mg/kg, the sample associated with that batch must be flagged or rerun.

12.7 The correlation coefficient of the calibration curve used to quantitate samples must be at least 0.990.

12.8 If any of the criteria above are not met, the problem must be corrected before further samples are analyzed. Any samples run between the last QC samples that meet the criteria and those that have fallen out should be rerun. If this is not possible that data must be flagged.

12.9 Solvent blanks should be run after samples suspected of being highly concentrated to prevent carryover.

12.10 One of the duplicate spikes must be run at the beginning of the set of 20 samples and the other spike should be run after samples have run on the instrument.

12.11 Pace Reporting Limit Standard (PRLS) – A standard prepared at the concentration of the Pace Reporting Limit. It is analyzed after the calibration and monthly thereafter, recovery 60-140% of true value. If outside the limits, reanalyze once. If still outside the limits, recalibrate

13. METHOD PERFORMANCE

13.1 There are several requirements that must be met to insure that this procedure generates accurate and reliable data. A general outline of requirements has been summarized below. Further specifications may be found in the Laboratory Quality Manual and specific Standard Operating Procedures.

13.1.1 The analyst must read and understand this procedure with written documentation maintained in his/her training file.

13.1.2 An initial demonstration of capability (IDC) must be performed per S-ALL-Q-020, *Orientation and Training Procedure*s. A record of the IDC will be maintained in his/her QA file with written authorization from the Laboratory Manager and Quality Manager.

13.1.3 An annual method detection limit (MDL) study will be completed per S-ALL-Q-004, *Method Detection Limit Studies*, for this method and whenever there is a major change in personnel or equipment. The results of these studies are retained in the quality assurance office.

13.1.4 Periodic performance evaluation (PE) samples are analyzed per S-ALL-Q-010, *PE/PT Program*, to demonstrate continuing competence. All results are stored in the QA office.

14. POLLUTION PREVENTION AND WASTE MANAGEMENT

14.1 Pollution prevention encompasses any technique or procedure that reduces or eliminates the quantity or toxicity of waste at the point of generation.

14.2 The quantity of chemicals purchased is based on expected usage during its shelf life and disposal cost of unused material. Actual reagent preparation volumes reflect anticipated usage and reagent stability.

14.3 Excess reagents, samples and method process wastes are characterized and disposed of in an acceptable manner. For further information on waste management consult the current version of ALL-S-002, *Waste Handling*.

14.4 The laboratory Chemical Hygiene Plan/Health and Safety Plan contains additional information on pollution prevention.

15. REFERENCES

15.1 Modified DRO, Method for Determining Diesel Range Organics Wisconsin DNR, WI-PUB-SW-141, September 1995

16. TABLES, DIAGRAMS, FLOWCHARTS, APPENDICES, ADDENDA ETC.

TABLE I

DIESEL COMPONENT STANDARD AND CONCENTRATIONS FOR BOTH RESTEK AND ACCUSTANDARD STOCK STANDARDS

Component	Concentration, ug/ml
Decane	2000
Dodecane	2000
Tetradecane	2000
Hexadecane	2000
Octadecane	2000
Eicosane	2000
Decosane	2000
Tetracosane	2000
Hexacosane	2000
Octacosane	2000
Total	20000 ug/ml

17. REVISIONS

Document Number	Reason for Change	Date
GB-O-019-Rev.00	Reformatted SOP body to Pace Analytical Format	13Apr2005
S-GB-O-019-Rev.01	Updated Sections 5, 6, 14 and 15 with current standard information Changed all references of EnChem to Pace throughout SOP. Updated Section 12.4.2 with sample filtration procedure	21Sept2007
S-GB-O-019-REV.02	Updated signature page. Section 2.2 changes PQL to 2.0 and 50 from 5.0 and 100. Deleted Section 7. Renumbered document. Section 9.5.3 – Added ICV Standard source. Section 10.1 – Update ICAL Standard concentrations. Section 10.3 – Added ICV criteria Section 11.4.1.9 – changed final volume to 1 mL from 2 mL. Section 11.4.2.1 – Added what to do if weight is not available. Section 11.4.3 – Changed injection volume from 2 uL to 1 uL. Section 11.5.2 – Changed Conifer references to Epic Pro. Section 11.5.4 – Changed Water final volume to 1 mL. Section 12.1 – Updated IDOC spike concentrations. Section 12.2 – Updated LCS Spike concentrations. Section 12.6 – Changed concentration from 2.5 to 2.0 mg/kg. Section 10.4 and 12.11 – added PRLS. Section 13 – Updated SOP references.	01Oct2008
S-GB-O-019-Rev.03	Updated Signature Page Section Section 11.4.2.1: Changed to see SOP: S-GB-O-018 DRO Weight Determination Section 11.4.2.4: Changed to Stir sample instead of shaking for 2 minutes.	18Mar2010